

nature

January 17, 1975

Should we leave it to the mystics?

THE French postal strike delayed until recently the arrival of the latest of one of UNESCO's more interesting periodicals, *Impact of Science on Society*. There are several journals attempting to fill the role of spanning the gaps between science, policymakers, educationalists and the public, and all of them, one suspects, suffer from the same problem: the scientist feels he should read them, but never quite finds time.

Impact (its long title sounds a little dull, its short one suggests it should be on the bookstalls along with *Probe*, *Thrust*, *Health* and . . . *Nature*) is always worth devoting time to, and this most recent issue, on the parasciences, is no exception. A dozen writers provide essays on subjects ranging from the design for a spaceship reported in the Book of the prophet Ezekiel to vision through the fingers. The whole is elegantly introduced through an essay (adapted from the *The Roots of Coincidence*) by Arthur Koestler.

One of the persistent complaints of those who work in fields outside orthodox science is that they cannot get scientists to take them seriously—although one must note that of 48 past presidents of the Society for Psychical Research, nine were Fellows of the Royal Society. And yet there are some, both scientists and parascientists, who believe that the two fields represent opposite sides of the coin and scientists should not be brought in to attempt to explain what is inexplicable in their terms (in much the same way that science and religion seem, on the whole, to have kept each other at a respectful arms-length). It is thus a matter of some interest to see what parascience says when given a highly respectable platform from which to speak to the establishment.

The result is somewhat disappointing. Part of this, no doubt, stems from the necessary variability produced in bringing together authors from many nations. But part of the disappointment is that, with the exception of Koestler, none of the proponents of the parasciences really gets down to the serious business of trying to persuade the reader that there is something in it.

Koestler is an exception because he can see the strange side of the modern physicist's perception of nature and he can play on it to create a certain malaise in the reader's mind. Time reversals, anti-matter, a hundred or more elementary particles—scientists can accept these, but still choke on the thought of extrasensory perception, let alone psychokinesis. It's a good though certainly not a clinching argument, but much of what follows in the journal demonstrates why it is that scientists are still sceptical. Practitioners either indulge in the production of wide-ranging samplers of superficial and inadequately reported observations, or plunge into extended deep speculation without so much as a glance at the reader to see if he

needs any convincing even to take the first step.

Yet for all this there are some good things, particularly where science and technology are allowed to do the explaining. Kirlian photography, the observation of corona discharges, is well dealt with by Rudolph Guzik, who makes it clear that a very complex (and in a sense unpredictable) phenomenon can be understood within the confines of 'normal science' provided that mystics, using words like 'life force' and 'psionic aura' do not enshroud the whole field in nonsenses and drive away those who could put it on a rational footing. One suspects that this is a paradigm for much of what exists in and beyond the fringe of science. There are many things which in the past were deemed not 'normal', that is not common-place experience. Among these could be included eclipses, earthquakes and mirages, and all of these have yielded to rational study. Nor is science reckoned to have failed because some of these phenomena are irregular and unpredictable as yet. Other 'not-normal' phenomena, being less easy to master scientifically and more susceptible to the attentions of quacks and tricksters, have now receded into a world of the half-light, and the scientist wishing to investigate has first to discern whether he is being taken for a ride by the practitioner. He may rather be taken for a ride by his own senses, and this indisputably is a subject worthy of research; a scientist may be able to satisfy himself that the practitioner is not consciously deceiving but be unqualified to spot his own perceptual errors. For a start he should be suspicious of any phenomena which require darkened rooms or his ability to absorb a wide variety of happenings and pronounce on the normality or otherwise of one of these—not necessarily the one he was concentrating on.

There may well remain, however, things worthy of our attention—a sort of dry residue. The parasciences may not yet have unearthed much for which there is a cast-iron case for scientific explanation, but there is ample material crying out for verification, and the lessons learnt over the last few years should have improved techniques and procedures for getting to the heart of the deception/reality question. Now, surely, is the time for scientists in large numbers to take a more practical interest in these obstinate issues. If we fail to do so, parascience will recede further into the mystic's world and will take with it much of the public's sympathy.

Investigation in the para-world is neither easy nor always agreeable. Public interest, however, demands, rightly or wrongly, that scientists come to grips with the propositions being made. And if scientists won't come in with rational attitudes and, where necessary, rational explanations, they can hardly be surprised if the forces of irrationalism take over. □



Any old Fe₂, Cu, Al, Pb, Zn, glass, paper. . . ?

The future availability and price of key raw materials, placed in doubt by the events of the past year, have emphasised the need to conserve by recycling. Attitudes in the UK are reflected in the following three articles. The first is by Christine Thomas, of Friends of the Earth.

IN modern times, industrial societies have been characterised by an attitude to the use of resources which earlier societies necessarily regarded as untenable. The difference in attitudes was born of a different appreciation of resource availability: in recent years industrialised regions have evolved a sophisticated system of resource-monopolisation and hence cost-control which enabled them for all practical purposes to regard raw materials as infinite or at least theirs for the taking.

This system and the attitudes which it bestowed are now breaking down for two reasons. First, there has emerged a greater maturity of view shared by many of the countries described alternatively as 'developing' or 'emergent', which have important raw material reserves. Second, the real resource scarcity predicted for certain commodities has begun to bite and has been reflected in the rising prices of raw materials.

It is therefore self-evident that the more efficient use of raw materials in 'developed' countries should become an important 'development' goal. Britain, a country more heavily dependent than most on the import of essential raw materials, can no longer afford to discard over 100 million tonnes of waste every year. Even if more efficient recycling systems and policies of re-use were instituted, Britain would still be dependent on raw material imports.

Recycling of industrial products is one option that can lead to a thriftier use of resources. By attempting to approximate to natural cycles, such as those for carbon and nitrogen, where no material is lost from the system, recycling can partially close the loop of industrial production. By doing so this decreases both the production of waste and the extraction of virgin raw materials associated with a given level of materials consumption.

Current practice though shows these loops to be far from closed. The table below illustrates the present use of reclaimed materials in Britain. The figures in the first column are deceiving as they include the use of 'new' scrap or manufacturers' scrap. More telling is the percentage of 'old' or 'post consumer' scrap recovered.



Material	% (by weight) of total production accounted for by:	
	All scrap	Old scrap
Iron and steel	52	10
Copper	40	13
Aluminium	30	—
Zinc	25	—
Lead	65	—
Glass containers	22	3
Paper	43	15-30

Comparison between the energy requirements for a number of metals produced from secondary materials and from their ores show that for the former the energy needs are invariably smaller. Often dramatically so, as with aluminium where the energy costs of its production from bauxite are 30 times that for its production from scrap. Recycling paper also offers potential energy savings, as production of fibreboard from virgin pulp requires about twice the amount of energy compared with its production from waste paper.

The British Steel Corporation accrue considerable savings through their recycling efforts. They used 19 million tonnes of scrap steel last year, nearly half their raw material requirements and with a 30% saving in energy associated with processing scrap this resulted in almost 80,000 kWh (thermal) saved.

Since it would seem desirable on many accounts to maximise our recycling effort, why is current performance so low? Basically it is a question

of economics. In many cases it does not pay the individual firm to recycle, even though it would be of benefit to the country as a whole. A situation therefore arises in a free market, in which industry can externalise the social and environmental cost of production, creating a situation where virgin material use does not reflect its true cost to society. Thus recycling suffers unreasonably in competition with raw materials.

One controlling factor which is often overlooked and which is of great importance in deciding what is a desirable level of recycling is energy. In many cases recycling would not be justified where the energy costs of recovering a material far exceed that of initial production. Generally this is not the case, but mixing materials together, particularly in small proportions, makes it energy expensive to reverse the process and recover one material.

An additional constraint on the achievements of recycling is imposed by our attempts to continue growth in the production of consumer goods. In a 'growth' situation, reclaimed material arising from the previous year's production cannot by definition meet this year's demand. And in a situation of exponential growth the gap between the total supply of materials required to meet demand and that met by recycled products will continue to diverge.

*The front rank of the scrap metal trade, where an infrastructure of merchants has been built up over many years to feed metals back to the smelters.
Picture by John Rigby.*



John Newell reports on a recycling system which is being operated at a government laboratory.

THE automated pilot rubbish sorting line is now working at the Department of Industry's Warren Spring Laboratory. And most of the data concerning the total municipal and industrial waste produced by the county of Oxfordshire has now been assimilated in a project at the same laboratory which is intended to estimate how much of the raw materials needs of such a typical area could be met by a wasteplex, a single, central, all-purpose recycling centre. Great interest has already been shown in the Warren Spring Waste Materials Exchange, which will act as an information service providing regular quarterly bulletins, shopping lists of waste products which could be just what are needed as raw materials by someone else. Although the Warren Spring Laboratory is now spending at least four times as much as they were five years ago on recycling engineering, their resources are still limited when compared with the USA. The members of the team, which has been trying—and now succeeding it seems—to interest industry in new recycling technology developed at the laboratory, can be excused some irritation with accusations in the media that they are partly responsible for sluggish British

interest in moving away from tipping and towards recycling of solid and other wastes.

The sorting line now occupies about half a hangar-sized shed. It is fed by six tons at a time of raw rubbish from Stevenage dustcarts. The rubbish goes through a bag burster and then a rotating drum, which sorts it through three successive sieves with increasingly large holes. The various conveyors coming off this take their loads past magnets, low powered fans, a water bath to separate light from heavy constituents, and devices where rubbish is flung off high speed conveyors against angle plates, or drums rotating in the opposite direction, to make use of differences in the coefficients of friction and resilience.

Out of the other end, or rather ends, come several potentially valuable by-products. Three grades of re-useable paper, two already pronounced as suitable for board making by commercial board mills, one suitable for high grade fuel; clean tin cans for remelting; vegetable matter for composting or conceivably for animal feed, coarse rags for floor covering materials and fine particle materials for soil conditioning.

Warren Spring has also developed means for separating glass to a very high degree of purity because, although there is little commercial future for recycled glass in high grade uses at

present, the position may change, and recent developments in glass fibre manufacture make it likely that this will soon become a large scale outlet for recycled glass.

The lowest grade, dirty, wet mixtures of plastic, paper and other organic unmentionables could at least provide heat, if distilled in a pyrolysis plant set up on a wasteplex site. Warren Spring finalised its designs for a pyrolysis plant earlier this year, after some years of research, and are now waiting for the go-ahead to build a commercial version for a local authority. It has also had fully developed for some time an ingenious fluidised-bed technique for separating different non-ferrous metals.

As a result of talks now in progress, Warren Spring hopes that a first rubbish recycling line, probably using not just a few elements but most or all of the different sorting techniques it has developed, will be built for a local authority somewhere in Britain within 12 months. There will probably be no need to scale up since the existing plant can cope with 50 tons every 24 hours—the equivalent of the rubbish output of a city of about 50,000 people.

Unlike rivals, the Warren Spring system keeps rubbish in large lumps as far along the line as possible. Shredding is a late stage. This prevents the transfer of dirt to paper and tin cans that are being squashed inwards into their contents, and generally makes sorting, especially of glass, much easier. The Warren Spring line uses relatively few low powered air fans, which cuts running costs and does away with the need for cyclone air purifiers.

Some 25,000 to 30,000 companies are being approached during the setting up of the waste materials exchange scheme, in which Warren Spring hopes to supplement the existing communication lines of this kind with their quarterly catalogue, which will list the more *outré* waste products, including a good many normally considered toxic but still potentially recoverable. One example has already been the reclaiming of seeds, surplus to requirement which had been given a dressing of a mercuric fungicide; this enabled them to be conveniently recycled into an adhesive for wallpaper, also requiring a fungicide.

Full scale wasteplexes including, as well as pyrolysis plants and sorting lines, specialised centres like that at Pontypool for the reclamation of toxic wastes, and plants for de-inking news print, are perhaps 10 or 15 years away in Britain. But the Oxfordshire study which began in August will soon provide detailed guidelines for such projects which are adaptable for a wide range of districts—Oxfordshire was chosen because of its many and various small industries and life styles.

T. S. McRoberts, Director of the Wolfson Recycle Unit, Queen Mary College, London, describes the background to the unit's work.

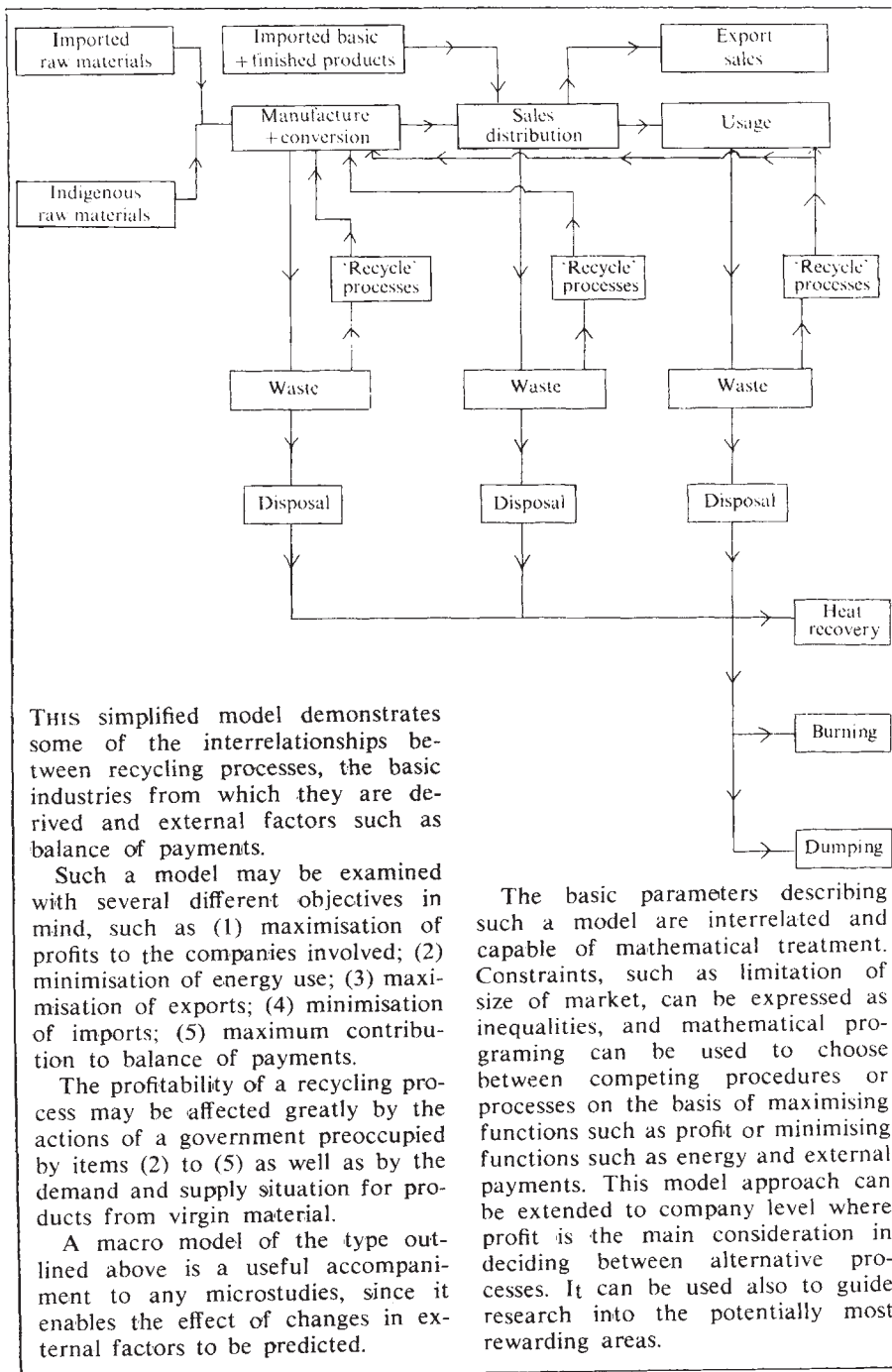
IN ANY study of recycling a great deal can be learnt from what industry is already doing. Many industries already carry out recycling in some form. The chemical industry operates large scale polymerisation plants where the economics of manufacture depends critically on the recycling of monomer. The efficient converter of plastics recovers and reuses offcuts and rejects from moulding operations. The UK paper and paperboard industry finds almost half of its feedstock in waste paper. Yet the bulk of plastics are consigned to municipal dumps after their first use and only about a third of paper is returned to mills for reconstitution and reuse.

Recycling of an obviously profitable nature is already being practised. The most notable example is the scrap metal trade, where an infrastructure of small and large merchants has been built up over many years to feed ferrous and non-ferrous metals back to the smelters. Only the more easily available and profitable sources are tapped, however, and it is estimated that 1 million tonnes of metal are discarded annually into domestic dustbins.

A major obstacle to the reuse of materials has been the cyclical nature of many key industries. At times when business is in the doldrums and mainstream plants are underloaded, prices of products from virgin materials are depressed close to marginal manufacturing costs and the waste recovery industry is immediately jeopardised. Among such industries are the plastics and paper industries, which are the subject of a technical and economic study by our unit.

Both the plastics and paper industries are now entering a recession. The comparatively new plastics recovery industry is under severe economic strain and waste paper merchants have large amounts of expensive working capital tied up in stocks which are proving difficult to sell.

While the waste recovery industry is preoccupied mainly with its own efficiency and profitability, the nation as a whole has a balance of payments crisis linked to heavy importation of energy in the form of oil. The UK imports most of its paper requirements in the form of pulp or finished paper; for example domestic wood pulp accounts for only one-sixth of its wood pulp requirements. The plastics industry depends almost entirely on feedstocks from oil, all of which is at present imported. It is obviously important from a national viewpoint that



THIS simplified model demonstrates some of the interrelationships between recycling processes, the basic industries from which they are derived and external factors such as balance of payments.

Such a model may be examined with several different objectives in mind, such as (1) maximisation of profits to the companies involved; (2) minimisation of energy use; (3) maximisation of exports; (4) minimisation of imports; (5) maximum contribution to balance of payments.

The profitability of a recycling process may be affected greatly by the actions of a government preoccupied by items (2) to (5) as well as by the demand and supply situation for products from virgin material.

A macro model of the type outlined above is a useful accompaniment to any microstudies, since it enables the effect of changes in external factors to be predicted.

the maximum amount of worthwhile recycling of both paper and plastics should take place. The future of recycling in these industries will depend to some extent on a successful formula being found for mitigating the worst effects of economic fluctuations in the primary industries.

The technology of recycling is influenced by the fact that both paper fibres and plastics materials deteriorate in quality in the course of both processing and reprocessing. Each time paper or paperboard is recycled its fibres become shorter and therefore less satisfactory for paper and paperboard products requiring strength. Although this does not appear yet as a limiting factor in recycling, it may well have to be

taken into account as more and more paper is recovered.

The problem of deterioration in quality and performance is much more serious in the case of plastics. Of the two major groups, thermosets and thermoplastics, only the latter can be considered seriously for recycling purposes. The largest outlet for thermoplastics is in packaging, which absorbs 35–40% of production and most of which appears as waste within weeks.

One would prefer to recycle a material back to the use from which it came, but if the material is degraded on reprocessing, so that it is not acceptable for its original use, a crucial question will be the size of the market for the degraded material. □

international news

A TOP-LEVEL advisory committee has told NASA that, unless the agency's dismal budgetary outlook improves, it should take a close look at its plans for planetary research and perhaps even drop or defer two important missions to the outer planets in the late 1970s. The panel makes that suggestion reluctantly because it believes that the expensive planetary flights could squeeze out cheaper, but equally important, missions.

The recommendation, contained in a report prepared by the Space Science Board (SSB) of the National Academy of Sciences, is one of several suggestions about what should be the priorities in space research in the coming years. The report is likely to be highly influential in shaping NASA's science programmes because it was produced by a panel of distinguished (read powerful) astronomers and space scientists with the help of a large number of university researchers. It will be published early in February by the academy.

Starting from the assumption that NASA's space science budget is unlikely to grow much, if at all, in the next few years—an assumption which is borne out by recent trends—the SSB has recommended what it calls a balanced programme by assigning priorities to missions which have already been approved by Congress and, more important, to missions which are still in the planning stage. Although the report says nothing about manned space flight or applications satellites, it assumes that the space shuttle will be developed to put large payloads into Earth orbit.

As far as approved projects are concerned, the SSB strongly supports the High Energy Astronomy Observatories, the Pioneer Venus project and the Mariner Jupiter-Saturn mission, all of which have been reduced in scope and delayed by budgetary restraints. Those three missions, the report says, "represent the next opportunities for important advances in space astronomy and in planetary and lunar exploration". The Pioneer-Venus programme, with the launch scheduled for 1978, is however, already in danger of being delayed by several months as a result of budget cuts proposed by President Ford in November last year; Congress has yet to act on those proposals.

As for projects which are still in the planning stage, the SSB recommends

A blueprint for space research

by Colin Norman, Washington

that a start should be made next year on the Large Space Telescope (LST). An optical telescope which would be flown on one of the early shuttle launches, the LST would, according to the SSB, be "an exciting major step in the history of observational astronomy, observational cosmology and the understanding of the Universe". But it would also be extremely expensive. Consequently, the report suggests that NASA should consider a rather less ambitious LST than it had originally planned to develop.

In short, the LST has been envisaged as a 3-m instrument, but the SSB suggests that it should be reduced to 2.4 m. Even that would, however, be able to detect stars ten times fainter than is possible using ground-based instruments, and it would also have a resolution ten times better. Given the bleak budgetary climate, NASA will probably be only too pleased to take the cut-rate option.

The LST would be the only new start for the next fiscal year, but the SSB suggests that NASA should begin developing hardware for eight other new missions in the fiscal year after that. They are a satellite which would be placed in polar orbit around the Moon, analysis of data from the 1976 Viking Mars Lander mission and planning of further Mars exploration, a spacecraft which would be sent into orbit around Jupiter and which would send probes into Jupiter's atmosphere, a spacecraft which would swing past Jupiter and travel on to Uranus, an orbiting telescope to take advantage of maximum solar activity in the early 1980s, a satellite for infrared astronomy, a gamma-ray satellite and finally, a spacecraft for electrodynamic studies.

Unfortunately, however, if all those projects are given the green light in the 1977 fiscal year, expenditures in subsequent years would probably break NASA's budgetary ceiling. The SSB therefore suggests that if NASA's

budget fails to increase, the agency should either delay developing and launching the Jupiter orbiter for four years or it should scrap plans for the mission to Jupiter and Uranus.

Although both of those projects have considerable support in the scientific community, the SSB says that if they are approved at the expense of high priority programmes in other fields, the space science programme would be unbalanced. Because the launch date of the Jupiter-Uranus spacecraft is tied to a rare alignment of the outer planets, that mission could not be delayed.

Between 1978 and 1982, the SSB recommends that NASA should begin work on a variety of other projects, including an imaging radar which would be placed in orbit around Venus, orbiters around Jupiter, Mercury and Saturn, a spacecraft to intercept the comet Encke, another infrared telescope, a 1.2 m orbiting X-ray telescope, and possibly a spacecraft which would travel out of the ecliptic plane.

The report makes clear that there is a difference of opinion among planetary scientists about the exploration of Mars after the 1976 Viking lander mission. The report recommends that a long term goal should be to bring samples of the surface of Mars back to Earth for analysis and inspection, but that the next stage of exploration should be to place a spacecraft in polar orbit around the planet, and to follow that with a probe and hard lander in the early 1980s. An SSB subcommittee on planetary research urged, however, that existing spacecraft should be modified so that another Viking lander could be despatched to Mars in 1981 if the 1976 mission produces particularly exciting results. But the SSB report said that keeping such an option open would be too expensive: "we see no missions . . . that should be displaced to meet such option costs", the report states.

The missions recommended by the SSB would keep NASA's space science budget essentially the same in terms of purchasing power over the next six or seven years, but with inflation running at its present rate and President Ford preaching fiscal restraint, it remains to be seen whether even that modest expectation will prove to be too optimistic. There should be some clues when the Administration publishes its 1976 budget recommendations on February 3. □

Canada adopts new nuclear safeguards

from David Spurgeon, Ottawa

STUNG into reconsidering its nuclear export policy by India's explosion of a nuclear device last year, Canada has announced that more stringent safeguards will now be applied to sales abroad of its nuclear technology, facilities and material. But, instead of calling for a complete ban on nuclear sales—as it once considered doing during a seven-month policy review—Canada has decided actually to encourage such sales provided new safeguards are met.

The decision has considerable importance. The Indian explosion (achieved with the unintended aid of a Canadian research reactor and personnel training programme) embarrassed government officials here just at a time when the CANDU reactor's remarkable success at home was beginning to pay off in export sales. By stirring Canadian consciences with the threat of further nuclear weapons proliferation, the Indian incident threatened to frustrate one of the country's few successful forays into high technology just when the superiority of CANDU was being proved.

To some observers, the prospect of Canada failing fully to exploit commercially a successful technology, after the expensive research and development had been accomplished with government funds, seemed balefully familiar.

The government's rationale for its new policy, announced on December 20 by the Minister of Energy, Mines and Resources, Donald S. Macdonald, was thus to place in balance the need for foreign trade of the Canadian economy, the need for cheap electrical power of developing countries at a time of world-wide oil shortage, and Canada's responsibility for ensuring that her nuclear resources do not contribute to the proliferation of nuclear weapons.

"The provisions, to be administered by the International Atomic Energy Agency, or through appropriate alternative procedures meeting the requirements of the Treaty on the Non-Proliferation of Nuclear Weapons", said Mr Macdonald, "will cover all nuclear facilities and equipment supplied by Canada for the life of those facilities and equipment."

"They will cover all nuclear facilities and equipment using Canadian-supplied technology. They will cover all nuclear material—uranium, thorium, plutonium, heavy water—supplied by Canada, and future generations of fissile material produced from or with these materials. They will cover all nuclear materials, whatever their origin, produced or processed in

facilities supplied by Canada."

"Most importantly, all safeguards arrangements will contain binding assurance that Canadian-supplied nuclear material, equipment and technology will not be used to produce a nuclear explosives device, whether the development of such a device be stated to be for peaceful purposes or not."

This latter remark was a reference to the Indian case, in which that country claimed not to have violated previous agreements with Canada because her nuclear explosive was for "peaceful purposes"—an interpretation never agreed to by Canada but now explicitly excluded for the future.

Potential Canadian exporters of nuclear material, equipment or technology were advised that in future they will have to ascertain from the government that there are no safeguards or impediments to sales before making offers of supply.

Having outlined the safeguards, Mr Macdonald said that, "to ensure that Canadians will enjoy the economic gains from sales abroad, the government will encourage the supply from Canada of major high technology components and services." It would also seek domestically to "establish a cooperative approach of preference (among the provinces) for Canadian material equipment and services."

The Canadian industry at present has the capacity to produce at least three nuclear reactor power systems a year, the minister said. Domestic requirements will average four units a year over the remainder of the decade, and exports could add at least one additional unit a year. Nearly \$100 million in capital investment has already been committed or planned by the private sector to expand capacity, and future demands will require perhaps \$100 million more. The federal Department of Industry, Trade and Commerce will see if it can help provide it through incentive programmes.

Meanwhile, the government has lifted the freeze put on nuclear exports after India's explosion and has authorised Atomic Energy of Canada Limited to negotiate a number of foreign sales and agreements, provided that they comply with the new safeguards.

These include agreements with the United Kingdom covering CANDU/SGHWR technological exchanges and heavy water supplies, a licensing agreement to supply CANDUs to Italy, goods and services estimated at \$150 million for a second 600-MW CANDU power station for Argentina, two 600-MW CANDUs for Iran—possibly four, one reactor for Korea, the nuclear part of a CANDU for Denmark, and technology agreements with Rumania.

Opposition parties have said that the new policy does not go far enough to prevent the spread of nuclear weapons. James Balfour, of the Progressive Conservatives, said nuclear agreements should not be signed unless the country involved allowed inspection of all its nuclear facilities, not just those acquired from Canada. This would prevent countries from simply copying a CANDU and using it to produce plutonium.

But Mr Macdonald claimed that the new policy makes Canada's safeguards standards higher than those of its competitors—and only time will tell if that will hurt sales. He said that International Atomic Energy Agency safeguards do not cover technology or prevent the use of nuclear materials for peaceful purposes, as Canada's now do.

In the last analysis, of course, everyone recognises that no form of international agreement will guarantee non-proliferation of nuclear weapons; that depends upon each nation's willingness to abide by the agreements. Mr Macdonald admitted as much outside the Commons after his announcement. And if trade partners with Canada decide to break their agreements, he said, Canada will have no recourse but to embargo future nuclear sales, as was done with India. □

Useful, unsurprising

THERE are no surprises in the recent report on energy conservation prepared by the National Economic Development Office (*Energy Conservation in the United Kingdom*, HMSO, £3.40) but a useful collection of relevant facts and figures which have been lacking in some other surveys.

As expected the report pinpoints domestic and office heating as the main areas in which savings can be made without too much social upheaval, and suggests a comprehensive analysis of the type of fuel and energy supply which would give the most efficient use of primary energy. The facts show that the net energy supply to the domestic market increased very little from 1960 to 1972, although the pattern of fuel use changed drastically. About 70% of this energy goes into space heating, and as the number of homes is steadily increasing and the population is changing to include a greater number of elderly people who will require higher living temperatures, the greatest savings in energy demand can be made here by a much more widespread use of proper insulation. An energy saving of 22% for a new three-bedroomed, semi-detached, centrally heated house could be achieved at an extra cost of a few hundred pounds a house. □

THE long awaited verdict on whether or not the artificial sweetener saccharin causes bladder cancers in rats may still be several years away. Last week, a committee of the National Academy of Sciences, which had been examining the evidence for two and a half years, said that there is not enough reliable data to state that saccharin is or is not a carcinogen. Consequently, the Food and Drug Administration (FDA) announced that it will do some more tests and continue to freeze consumption of the sweetener at 1972 levels.

The academy's report, which presents a detailed analysis of the history and chemistry of saccharin as well as a searching critique of the carcinogenicity tests, leads, however, to two disturbing conclusions. The first is that there has been a scandalous lack of study by the FDA of a chemical which is consumed by about 12 million people in the United States, at the rate of some five million pounds a year. And the second is that most of the saccharin produced in the United States is contaminated by an impurity, *o*-toluenesulphonamide (OTS), which could itself be a carcinogen. The latter possibility may not only have distorted carcinogenicity tests on saccharin, but it also raises the question of why the FDA has not taken steps to determine the biological significance of OTS and, if necessary, to ensure that the sweetener is manufactured in such a way that the impurity is kept out.

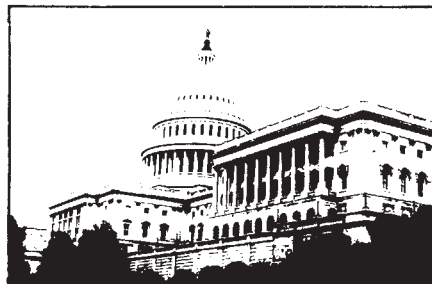
If OTS is, indeed, a carcinogen when fed to animals, the so-called Delaney amendment to the Food and Drug Laws would require that saccharin contaminated by OTS must be banned from the market. Saccharin is manufactured by two commercial processes, the most widely used of which produced OTS impurities at the level of several hundred parts per million, whereas the other results in contamination of only a few parts per million.

There have been two principal studies which raised fears that saccharin may cause bladder tumours in rats. One was carried out by the FDA itself and the other by the Wisconsin Alumni Research Foundation (WARF)—a laboratory supported by the sugar industry. Both studies revealed increases in the incidence of bladder tumours in rats fed saccharin at doses of 7.5% and 5% of the daily diet. The sweetener was administered to pregnant rats so that their offspring were exposed before birth, through the mother's milk and subsequently in their own diets.

The academy committee points out, however, that because the rats were also getting OTS "at very large dose levels", it is "impossible to say whether

saccharin itself was the carcinogen". The report also raises the question of whether bladder stones and parasites may have played a role in the formation of tumours. But, on the other hand, the academy report notes that most of the tests which have produced no evidence of carcinogenicity did not involve exposure to saccharin *in utero*. Therefore, they "cannot be interpreted as showing that saccharin is not a bladder tumorigen".

The FDA study and the WARF study were completed early in 1973,



Washington seen

by Colin Norman

but an FDA scientist acknowledged last week that the agency has sponsored no more tests since then, even though doubts have been raised about the role of OTS. The academy therefore recommends a series of studies to investigate the significance of impurities in commercial saccharin and the role of parasites and bladder stones in tumour formation, and epidemiologic studies to see whether there is any relation between cancer and long term consumption of saccharin in man. Dr. Julius Coon, of the Thomas Jefferson University in Philadelphia, who was the chairman of the academy committee, said last week that the tests could take about two years to complete.

● No action of Mr Nixon's upset the elders of science in the United States more universally than his decision to shift responsibility for formulating science advice from the White House to the bureaucratic hinterlands of the National Science Foundation. President Ford's announcement last month that he has set Vice-President Rockefeller the task of determining what arrangements should be made for getting science advice to the President was therefore greeted with considerable enthusiasm. But, with Rockefeller occupied in trying to determine the truth of allegations that the CIA indulged in domestic activities, the operation has taken something of a back seat. Henry Diamond, a lawyer connected with Mr Rockefeller's Critical Choices Commission in New York, has been given primary responsibility for the study and some announcement is expected

within the next month or so.

A number of groups, including the National Academy of Sciences and the US Senate, have publicly endorsed the view that a science policy apparatus should be re-established in the White House, and it seems likely that the Rockefeller study will recommend such a move. A key issue, however, will be the extent to which the mechanism will have influence over military research and development — when Nixon scrapped the old arrangement he specifically told the National Science Foundation to keep out of military affairs. And another central factor is how it will interact with the Office of Management and Budget. It is understood that those questions are now being debated among Rockefeller's advisers.

● A Boston physician went on trial last week faced with a manslaughter charge for allegedly killing a foetus during an abortion he performed last year. The trial has drawn considerable attention throughout the United States because it is likely to define in more precise terms when a foetus should be considered capable of surviving independently from its mother.

The physician, Dr Kenneth Edelin, was charged last year by a grand jury of aborting a foetus—described in the charge as a "baby boy"—which was believed to be between 24 and 28 weeks' old, and of failing to take steps to assure its survival.

When the Supreme Court handed down its historic decision on abortion two years ago, it ruled that state laws cannot forbid abortions before the foetus is 'viable', but it deliberately left fuzzy the definition of what constitutes viability. Its decision, in fact, is a model of obfuscation, since it states that viability "is usually placed at about seven months but may occur earlier, even at 24 weeks". The foetus that Edelin aborted is right in that uncertain period, and the trial is therefore likely to hang on the definition of viability.

But, aside from defining more precisely the scope of the Supreme Court's abortion decision, the trial could also have some implications for foetal research. There have, during the past year or so, been several state and national laws enacted which attempt to put a stop to research involving so-called living foetuses; but those laws have largely been passed in the absence of any definition of what constitutes a living foetus. They are generally regarded as greatly restricting some important areas of biomedical research.

The trial is expected to last for four or five weeks, and Edelin could face a lengthy prison term if found guilty.

Moves to set up a register of toxic chemicals

from Peter Collins, Geneva

THE first practical steps towards setting up an international register of potentially toxic chemicals were taken at a meeting at Bilthoven, The Netherlands, this month. Sponsored by the United Nations Environment Programme (UNEP), and hosted by the Netherlands Government, the meeting marks an unexpected change of mood on the part of governments in their approach to a subject which, when raised at the preparatory committee for the Stockholm Conference on the Human Environment, seemed one of those on which early action was least likely. During the committee's discussions the suggestion for such a register had a consistently lukewarm reception, especially from those governments most able to contribute to it. This was largely because of a feeling that so many sensitive areas of industrial confidentiality and, probably, military security were involved that real co-operation on the part of both manufacturers and governments—as users of many candidate materials—would be virtually impossible to achieve.

There has evidently been a big change of attitude, especially on the part of governments, though not yet so visibly on the industrial side. Perhaps the principal reason for this is the sheer pressure under which governments have found themselves when it comes to testing the enormous number of new potentially toxic substances being produced each year, the position of which needs to be defined with regard to the increasingly large and strict body of legislation in many countries, itself in part a result of pressure arising from the atmosphere of the Stockholm Conference.

Governments, it would seem, no longer have the same attitude to confidentiality over much of this field as they did two or three years ago. The more details are made public, the greater is the chance of making widespread testing of potentially dangerous new products more effective; if information is more widely available, testing to acceptable standards can be carried out cooperatively, and the number of candidate substances covered in a given time can be greatly increased. Governments, in fact, are facing a situation in which they cannot, working individually, keep up with their own requirements, since testing to modern standards is so lengthy and costly a process, and a situation already exists

where virtually all the testing is being done in a small group of countries, working informally on behalf of a much larger number of governments. Any facility to help the pooling of information, such as could be provided by an international register, would at least tend to avoid duplication.

As regards industry, it would seem that the gloomy view of the prospects of collaboration at the time of the Stockholm Conference was based on a misconception. The problem is not that industry keeps quiet because it has so much dangerous information to hide. In fact, industry does very little testing beyond a certain point at present, leaving the finer details to governments. What could well be hidden by industry, the UNEP believes, is ignorance of the toxic properties of its own products, rather than information about known dangers. It looks almost as if many companies are keeping quiet because they know so little, not too much.

For these reasons, the proposed register is predicted by the UNEP not on industrial information, but rather on that which is already, and which will be needed, on an international exchange basis. Here again, governments remain the key to the provision of information on which to base any realistic international register.

Some national, and even international, registers do already exist. They vary widely in scope and detail, and help to show up some of the problems that will be involved in any project such as the one the UNEP has in mind. Thus, a list of toxic substances published in the US for the year 1973 runs to nearly 1,000 pages and covers more than 10,000 preparations—none of them described in such detail as the UNEP believes to be necessary for the sort of register it is aiming at. At the other end of the scale come the monographs put out by the International Agency for Cancer Research at Lyons, which now covers more than 100 compounds. These are as complete as could be wished.

In the UK, thinking on how to respond to the UNEP initiative is well advanced. Acting on the advice of the Royal Commission on Environmental Pollution, and of the Royal Society, the Department of the Environment has engaged the UK Chemical Information Service to design and develop a Network on Environmentally Significant Chemicals—DESCNET. This will be so designed as to provide input to, and to draw on, the UNEP International Register. In general, however, the approach in the UK will be selective and will utilise existing systems and data wherever possible.

Meanwhile, the UNEP feels that response to its initiative is widespread. Especially gratifying has been that from

the developing countries, which have a huge problem, inasmuch as they are not, with one or two exceptions, in a position to do any testing for themselves, and have to rely on what information they can gather from published reports or private enquiries elsewhere. The presence of representatives of the Soviet Union and the East European countries, however, perhaps interests the UNEP more at this stage, since there is the hope that from them, at least, information from industry may be available—as it should be, all industry being under government control.

The present meeting differs from previous consultations in this field in another respect: the majority of participants are people actively working on the problems of toxicity or in allied fields, or already concerned in compiling national registers, rather than official government representatives as scientific or administrative civil servants.

Moreover, the common denominator at Bilthoven is the chemical *per se*, not “the chemical in the environment”—in fact, an almost essential approach if the discussion, and the register that is its eventual objective, is to deal with the potential toxicity of new compounds, as well as with the hazards known to attend the use of those already in production. The aim, if the proposed register is to fulfill its purpose, is to satisfy the need for the unambiguous identification of chemicals, and of the dangers that may attend their use, if only because, unless such information is available, it is not possible to computerise the data—and the amount of information will certainly be such that it cannot be handled in any other way. So the intention is not just to list substances that are, or may be, released to the environment, but all sorts of potentially toxic substances, regardless of their categories of use.

Finally, although industry may at present seem to be reacting rather coyly to the idea of such a register, there is little doubt of a basic willingness to collaborate. This is not only because most companies involved in the production of candidate substances are anxious to improve their public image in this respect. It is also evident that while not at present likely to be providers of much information for the register (largely because they do not have the facts required), they will certainly be among the most concerned users of its outputs. The important thing is that we now have the chance of developing an orderly mechanism, internationally and nationally, for assembling an up-to-date dossier on chemical substances as a basis for environmental management and decision making. □

correspondence

Czech persecution

SIR,—I wish to raise some points about Professor Burhop's letter (November 22). Does he know about the existence of a law in Czechoslovakia, which is much more iniquitous than the one which may be accepted in West Germany? This is the Labour Code (law 42/1970), which contains section 46, lett. c, according to which a notice could be given to anyone who "violates the socialist social order". In the past five or six years tens (if not hundreds) of thousands of Czechoslovak citizens have lost jobs not only at schools, universities and justice offices, but also in the research institutes, mass media, theatres, publishing houses, museums, state administrative departments and so on because of the application of this paragraph.

Having been dismissed myself from the Nuclear Research Institute in 1970 under this paragraph, I tried to get revocation of my notice through the law. I shall quote here from the decision of the Court of Appeal, the highest authority in such cases:

"The claimant's activity was violating the socialist social order . . . as he signed a resolution . . . which demanded a change in the system of the leading organs (of the Academy) . . . Furthermore, the claimant tried to damage the development of educational and cultural policy of our state, which is led and directed by principles of Marxist-Leninist ideology, and this he did for instance . . . when he pleaded for the abolition of censorship of scientific publications."

Does the World Federation of Scientific Workers raise its voice against this law, which has been in force in Czechoslovakia for many years?

Professor Burhop's version of the shameful persecution of Malek is incorrect. Section 11 of the Czechoslovak pension law states that "persons who have reached the age of at least 60 enjoy a right to be retired". No obligatory retirement age is given in the law about the Czechoslovak Academy of Sciences (54/1963).

Professor Malek's contract with the academy had not been extended in October 1973, and Malek had either to find a new job or to retire (in 1970, as a measure of political persecution, the time limited labour contracts had been introduced in the Czechoslovak Academy of Sciences—20, 4, law

26/1970—for *senior* scientists only. The duration of contracts ranges from three months to four years). This dismissal has been an act of purely political persecution or even revenge. In contradiction with world academic habits, he does not enjoy the right to keep his office or laboratory in his former institute; he is now not even allowed to use the institute library.

I agree completely with Burhop's statement that issues like the retirement age should be "matters for the people of that country to decide". Is he not aware of the fact that the people of my country lost the right to decide on their own fate on August 21, 1968, when our country was invaded by foreign troops which were occupying my homeland?

The plight of Czechoslovak scholars and intellectuals is a direct consequence of this. One should not forget it.

F. JANOUCH

Copenhagen, Denmark

SIR,—I would like to comment on the discussion between Professors Janouch and Burhop (August 9 and September 20) concerning the situation of scientific workers in Czechoslovakia.

I absolutely agree with Janouch that "only open, public stands, protests and statements are meaningful and helpful". This is not only based on my own experience while living under this regime but also on experience during work with other institutions, such as Amnesty International. There is no doubt that the procedure suggested by Burhop might be effective with some regimes which respect—at least to some extent—reasonably open discussion. This is, however, certainly not the case in the so-called people's democracies, where the hypocritical Party bureaucrats not only suppress any sort of criticism but do not even respect their own constitutions and laws.

To illustrate the absurdity of the present situation of scientists in Czechoslovakia, I would like to bring up one flagrant example concerning Mr Karel Kriz, a former senior lecturer at the Technical University at Brno (Moravia). Like many of his colleagues, he was dismissed from his position because he declined to accept the Russian occupation as brotherly help. For some time he was not able to find any reasonable employment; in 1972, however, he started a "new career" as a buyer dealing in rabbit skins for the national enterprise KARA. Since this job was

not very well paid, he was compelled to organise his business using (for Czechoslovakia) new commercial methods: he sent various posters and information leaflets (on the eminent importance of rabbit skins for the national economy and so on) to all villages in the southern part of Moravia and informed the inhabitants of his impending arrival. In fact, he organised a group of gypsies, who regularly visited all villages and bought up rabbit skins for him and therefore for KARA. One of his posters read:

Dear friends.

KARA purchases hides and skins of every description. I shall be calling at your village once a fortnight. Kindly have your goods ready for me.

Thank you for your cooperation.

Your skin buyer.

Independent scientific worker

Doc. Ing. Karel Kriz, Candidate of Sciences, skin buyer for KARA, national enterprise.

His action soon became a great success, and the gypsies, villagers and KARA were exceedingly satisfied. Even the Party bureaucrats paid tribute to the successful gypsy brigade, which was soon accepted as one of the best "brigades of socialistic labour".

The disaster came in the summer of 1973 in the form of a panel discussion on Austrian Television devoted to the brain drain. At the end of the discussion, in which the Prime Minister, Dr Bruno Kreisky, also took part, the commentator said (ironically) that some neighbouring countries had succeeded in solving this problem better than Austria had. At the same time he showed Mr Kriz's poster.

Two days after the panel discussion, Mr Kriz was arrested on a charge of damaging the interests of the Republic, according to Section 112 of the Penal Code ("A Czechoslovak citizen . . . who damages the interests of the Republic by spreading or enabling to spread in other countries false reports about conditions in the Republic . . . will be punished with prison up to three years . . ."). The first trial, which should have taken place in November 1973, was postponed, and in the autumn of 1974 Mr Kriz was sentenced to three years in prison, the maximum punishment. What for? After all, the text of the posters was

approved by the censor and according to the law, nothing can prevent Mr Kriz from using his scientific degrees.

I would like to end by appealing to all scientists who are lucky enough to live in free democratic countries: please, do help actively our colleagues who are deprived of their human rights and are kept in prisons or are dying slowly in concentration camps and psychiatric hospitals, no matter where. If we do not help them, nobody will. After all, passive humanitarianism helps no-one except the totalitarian regimes.

E. ANTONCIK

University of Aarhus, Denmark

Trieste centre

SIR,—We must indeed be grateful for the letter from Sir David Martin and Mr Cozens (December 20/27) on the policy of the Royal Society UNESCO committee concerning the International Centre for Theoretical Physics (ICTP) at Trieste. Many of us who have been closely connected with the scientific programmes of the ICTP were relieved to learn that the British delegate to the recent UNESCO General Conference praised the centre, and voted for the full proposed financial contribution from UNESCO for 1975 and 1976. To that extent, at least, we have succeeded in our efforts (such as the meeting of the Royal Society on May 16, 1974) to ensure that this committee is fully informed about the work of the centre and is no longer taking decisions without reference to ascertainable opinion amongst British physicists.

The last paragraph of this letter further invites comment on the policy with which the British UNESCO delegation should approach the proposed review of "UNESCO's relations with and subvention to the centre". It is to be hoped that this policy will not be decided without thorough consultation, by the whole committee, with those who understand the situation at Trieste.

A kite is being flown, for example, that the ICTP could be financed mainly by direct contributions from individual governments or national scientific organisations, thus taking the burden from United Nations agencies such as UNESCO and the International Atomic Energy Agency (IAEA). This would be a disaster. As I thought was obvious from my article in *Nature* on March 22, the administrative resources of the ICTP are quite inadequate to the solicitation and collection of such contributions. It is able to carry out a valuable scientific programme on behalf of all nations, without serious political disruption, because it is a technical instrument through which a small number of international agencies (including the UN Development Program and the Swedish International Development Agency) channel their

efforts. Additional contributions from individual governmental organisations, such as Britain's Science Research Council, are very welcome, and are not inappropriate considering the scientific work that is actually done at Trieste by physicists from Britain and other advanced countries, but there is no substitute for the basic budget, shared by UNESCO, the IAEA and the Italian Government, that keeps the centre in active being. The real questions, rather, are the formal place of the contribution to the ICTP in the UNESCO budget, and the administrative devices by which it is maintained as a permanent institution within both UNESCO and the IAEA.

In view of the time and trouble it has taken in the past to get the facts about Trieste across to the British UNESCO delegation, it is not too early to air these issues in your columns (or in some suitably ventilated corridor!) in preparation for a wise decision in 1975 or 1976.

JOHN ZIMAN

University of Bristol

Hungarian attitudes

SIR,—Dr Peller's Hungarian experience (December 13) will only surprise those who believe that Hungary is a 'liberal' east-block state. In a very restricted sense this is true, but life for research workers in Hungary is difficult, and especially so if they intend to travel westwards. Outward evidence of the internal situation is shown by the difficulties Western scientists sometimes encounter if they want to travel in Hungary. As a former coworker in a research institute in Budapest, I had the opportunity of participating in the Conference of Solid State Physics held in Manchester in January 1966. I also intended to make a contribution and, as is the usual case in Hungary, I should have received by passport just one day before my travel. On that very day the Ministry for Metallurgical and Engineering Industry, my 'higher' authority, told me that I would not get a passport. No reason for this decision was given. This was only one typical case among several similar ones during the 17 years of my scientific career in Hungary. In general, for a young scientist, professional travels to Western countries are possible only if he is 'politically reliable'.

Undoubtedly only few entry applications are rejected. Israelis are especially not welcome, probably because of the great number of Jews living in Budapest, who might get authentic information on the real situation in the Middle East. Western scientists who have participated in a conference in Hungary, however, are in general delighted by the traditional hospitality of the organisers and this

helps to mask the autocratic treatment of the international scientific communities by the authorities.

LAJOS ERNST

West Berlin

University consultancy

SIR,—I would like to support Mr H. A. Cook's letter (December 13). Although I am not certain that all consultancy by university staff should be discouraged, for after all this is one way in which the results of research are transferred to industry, I am more particularly concerned at the use of university facilities for the production of hardware for commercial purposes.

In this company we are aware of at least two instances in which companies have been formed by university staff to manufacture equipment which is in direct competition with some of our own products. We are also aware that these companies have no manufacturing facilities of their own and that university plant, equipment and man-hours are used in the production of this equipment.

In effect, such companies have extremely small overheads which makes competition very difficult for a company like ours where all the research and development work must be paid for from profits.

I agree completely with Mr Cook that the appropriate government department should put a stop to the extracurricular activities of those university staff who, in effect, are being paid two salaries for doing one job and are using public funds under false pretences.

J. B. S. PRICE

Grubb Parsons,

Newcastle upon Tyne, UK

Periodic unification

SIR,—In your issue of October 25, page 661, paragraph three, there appeared an anonymous quotation and comment about the reception of some of Mr Edward Haskell's work, referring to a conference held in New York in 1971. The item: "'We had to listen to some extraordinary ideas of unification by way of Mendeleev's periodic table' said one participant. Another was horrified that whilst the scientists thought it silly, the philosophers present took it very seriously." I wish to set the record straight. There may have been more than one scientist who thought Haskell's work silly—blindness that was found in attitudes of some scientists towards precursors of Mendeleev's table. I can, however, certify that not only the philosophers present but also a number of highly qualified scientists who were also there, and who read Haskell's work, take it very seriously indeed.

HAROLD G. CASSIDY

Texas Christian University, Fort Worth

news and views

Protein turnover in the whole body

from J. C. Waterlow

In this issue of *Nature* (page 192) Young and co-workers at the Massachusetts Institute of Technology report measurements of the rate of protein synthesis in the whole body in human subjects from birth to old age. This is a timely contribution to a topic which is attracting increasing attention, and which has lain fallow for some time. It is 35 years since Schoenheimer, Ratner and Rittenberg (*J. biol. Chem.*, **127**, 333; 1939) reported "the first experiment in which one amino acid of a normal diet, tyrosine, has been labelled by the nitrogen isotope". In subsequent papers the concept of protein turnover was firmly established, but it was not for another 10 years that attempts were made by two groups, that of Rittenberg at Columbia and of Wu in Alabama, to measure the overall rate of this process in the whole body (Sprinson and Rittenberg, *J. biol. Chem.*, **180**, 715; 1949; Wu and Snyderman, *J. gen. Physiol.*, **34**, 339; 1950). In these early studies the method used was to give a single dose of an amino acid labelled with ^{15}N and to calculate the rate of turnover from the curve of ^{15}N excretion over the next 48 hours or so. It was soon realised that such a calculation is erroneous: the shape of the early part of the excretion curve is the resultant of a large number of processes, and cannot be analysed in a meaningful way. After the first few hours the slope is determined largely by the turnover rate of urea and not by that of protein.

From this point development occurred in two directions. Olesen *et al.* (Olesen, Heilskor and Schønheyder, *Biochim. biophys. Acta*, **15**, 95; 1954) measured ^{15}N excretion in urine for 15 days after a single dose of ^{15}N -glycine, and calculated the rate of protein synthesis by multi-compartmental analysis. Theoretically this method is an improvement on the earlier ones; in practice it has not been followed up, presumably because of the difficulty of maintaining a constant nitrogen intake and a steady state in subjects other than healthy volunteers. San Pietro and Rittenberg (*J. biol. Chem.*, **201**, 457; 1953) took an opposite line. They devised a method which depends on measuring the maximum ^{15}N abundance in urinary urea after a single dose of ^{15}N . Peak labelling occurs about 3 hours after the dose, and the calculation of

protein turnover depends upon this interval being accurately timed. In fact it is impossible, from measurements on urine, to know precisely the time at which maximally labelled urea was synthesised. The method is therefore unsound. In spite of this it was quite widely adopted in subsequent years to study protein turnover in various pathological states such as pituitary and thyroid disease. But the results in normal subjects showed a wide scatter, and nothing of interest was revealed in patients with metabolic disease. Probably for this reason interest in the subject waned. It is unfortunate that the method most widely used was the least reliable.

Nothing more seems to have been attempted for about 10 years until Picou and Taylor-Roberts (*Clin. Sci.*, **36**, 283; 1969) working on malnourished infants in Jamaica, used a continuous infusion of ^{15}N -glycine to produce an isotopic steady state. If nitrogen balance is measured as well, total rates of both synthesis and breakdown can be calculated. This is the method which six years later has been used to good effect by the workers at MIT. It probably gives more accurate estimates of total protein turnover than the older methods, but problems remain. On theoretical grounds a likely source of error is that even in the steady state the labelling of amino acids in the precursor pool for urea synthesis may not be the same as that of amino acids for protein synthesis. The report of Young *et al.* suggests however that the method gives results which at least have comparative meaning. Moreover, the value for protein turnover in normal adults, of $3 \text{ g kg}^{-1} \text{ d}^{-1}$ agrees well with results obtained by infusion of radioactive amino acids, whereby the special problems associated with urea formation are avoided (James, Sender, Garlick and Waterlow, unpublished).

It seems reasonable to suppose that

the difference in turnover rate in infants and adults is related at least in part to growth. A major contributor to whole body turnover is the turnover rate of muscle protein. Table 1, from unpublished results of D. J. Millward, shows the rates of synthesis and breakdown of muscle protein in rats of various ages. The difference represents net growth of muscle protein. Rapid rates of growth are accompanied by rapid rates of turnover. From the energetic point of view this is a costly process. The energy cost of protein synthesis is not negligible; estimates range from about 4 kJ per gram, based on the requirement of 4 moles ATP+GTP per mole peptide bond, to about 30 kJ per gram, derived from nutritional measurements. Whatever the true figure *in vivo*, it looks as if protein turnover must account for a considerable part of the basal energy expenditure. A general relationship was suggested between rates of protein turnover and of basal metabolism (Waterlow, *Lancet*, **ii**, 1091; 1968) and Young's work confirms this.

There remains a substantial gap between rates of protein synthesis at the subcellular level and in the whole animal. The information that is becoming available about synthesis rates *in vivo* shows that the efficiency of synthesis (g protein synthesised per g ribosomal RNA) in subcellular systems is perhaps 100 times less than the efficiency in the whole animal (Henshaw *et al.*, *J. biol. Chem.*, **246**, 436; 1971; Millward *et al.*, *Nature*, **241**, 204; 1973). Until this difference is reduced, we can rely on subcellular systems for information about mechanisms but not about rates. A major problem for the future is to understand the means by which rates of protein turnover are controlled. It is perhaps provocative to suggest that, in spite of all the difficulties, a solution is most likely to come from studies on the whole animal.

Table 1 Rates of muscle protein synthesis, breakdown and net growth in normal hooded rats at different ages

Age (d)	Fractional rate (% d^{-1})			Efficiency of synthesis (g protein/g RNA/d)
	Synthesis	Breakdown	Net growth	
23	28.6	22.4	6.2	19.2
46	16.1	13.1	3.0	14.2
65	11.5	9.8	1.7	13.8
130	5.3	4.6	0.7	10.1
330	4.9	4.6	0.3	11.5

Antibody structure and antigen binding

from C. C. F. Blake

ONE of the major problems in immunology has been the reconciliation of the structure of antibodies with the vast range of antigen binding properties they possess. The long and patient study of antibody structure by chemical methods has produced a basic framework for the solution of the problem by showing that antibody molecules have the following features: (1) the molecules are built from two identical heavy chains of ~440 residues and two light chains of ~220 residues, linked together by disulphide bridges; (2) there are four repeating homology regions or domains of ~110 residues in the heavy chains, and two in the light chains; (3) the amino-acid sequence of the N-terminal domains is variable; (4) the sequence in the rest of the molecule is more constant and allows the chains to be characterised as κ and λ light chains which can associate randomly with α , γ and μ heavy chains to give respectively the IgA, IgG and IgM major isotypic classes of antibody molecules; (5) within the variable sequence there are hypervariable regions. This information has been seen to imply that antibody molecules have a common basic structure, in which the antigen binding function is carried by the variable domain whose hypervariable regions are involved in the binding process.

For several years protein crystallographers have been seeking to give this elegant and satisfying model its final structural definition, and at the same time to provide a molecular description of the binding site and its mode of interaction with antigen. As with other studies in this field the work has been hindered by the heterogeneous nature of antibodies and to some extent by the size of the molecule. The former problem has been overcome in the usual way by using myeloma proteins, and the latter, although preliminary X-ray studies on whole immunoglobulins have been reported, by utilising fragments of the molecule; either the papain-induced Fab fragment which contains the binding site, or the naturally occurring Bence-Jones protein, a dimer of the light chains.

In the last year or so, four groups have independently reported high resolution structural analyses of these fragments. Poljak and his colleagues have worked on the Fab fragment of human IgG1 New (Poljak *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3305; 1973; Poljak *et al.*, *ibid.*, **71**, 3440; 1974), Davies and his group on a similar fragment of mouse IgA McPC 603 (Padlan *et al.*, *Nature new Biol.*, **245**, 165; 1973; Segal *et al.*, *Proc. natn. Acad. Sci., U.S.A.*, **71**, 4298; 1974) and the groups led by Edmundson (Schiffer *et al.*, *Biochemistry*, **12**, 4620; 1973) and Huber (Epp *et al.*, *Eur. J. Biochem.*, **45**, 513; 1974) have each solved

a human Bence-Jones protein. In confirmation of the chemical model all four groups report essentially similar tertiary and quaternary structures.

In the X-ray models the homology domains of ~110 residues appear like beads threaded on a string. Each domain has a tertiary structure that is basically two parallel β sheets between which is the hydrophobic core. The intrachain disulphide bridge, which is a characteristic of each domain, links strands in opposite sheets so that the two sulphurs are located near the centre of the domains. In the constant domain one sheet has four strands and the other three; the variable domain has an additional loop that extends the three-stranded β sheet of the constant domain to five strands. The link between the two domains is made by a short extended chain segment called the 'switch' region.

Each domain associates non-covalently with an equivalent domain in the other chain: the Fab has $C_{H1}C_L$ and V_HV_L dimers, and the Bence-Jones C_LC_L and V_LV_L dimers. This domain dimerisation has two unexpected features. The constant domains associate so that the four-chain sheets are closely opposed and their strands approximately perpendicular, but the variable domains oppose the other β sheet (the one with five strands) which leads to a looser association and a greater intersheet spacing. In the association each domain is related to the other in the pair by pseudo two-fold symmetry, but all four groups have found that the axes of symmetry between the pairs of constant and variable domains are far from colinear; angles of intersection between 120° and 135° are reported. As a result the switch regions bend through different angles in the two chains to give the four domains a tetrahedral arrangement. Although this is not altogether surprising in the Fab where the four domains are chemically distinct, the lack of structural equivalence between the chemically identical chains of the Bence-Jones proteins has led Schiffer *et al.* to suggest that one of the two light chains in the molecule fulfils the structural role of the heavy chain in Fab. This suggests that Bence-Jones proteins are counterparts of the antigen binding regions of immunoglobulins and may themselves have functional binding sites.

The close similarity of tertiary and quaternary structure observed in the Fab fragments and the Bence-Jones proteins strongly implies the invariance of many features in the architecture of antibody molecules, and allows some important predictions to be made. The amino acid homology between the C_{H2} and C_{H3} domains of the Fc fragment and the C_{H1} domain of Fab makes it

highly probable that the X-ray analysis of Fc now under way will reveal an equivalent structural homology. Poljak has shown from his results that the intrachain disulphide bridge in human γ_2 , γ_3 , γ_2 and μ chains, which is shifted by 83 residues on the heavy chain from its position in the γ_1 heavy chain in his molecule, can be made without altering the observed folding of the polypeptide chains. He also shows that a number of additional or unusual disulphide bridges which have been observed are made between residues that are already in the appropriate orientation. This is additional strong evidence that the results we already have in the Fab parts of the molecule provide the structural basis for all other classes of antibody molecule.

The properties of the antigen binding sites of the two Fab fragments have been investigated by binding their appropriate haptens: vitamin K to Fab New (Amzel *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1427; 1974) and phosphorylcholine to Fab McPC 603 (Segal *et al.*, *ibid.*, **71**, 4298; 1974). In addition Edmundson, following his suggestion of the analogy of Bence-Jones proteins to Fab, has examined the binding of dinitrophenyl ligands to his protein (Edmundson *et al.*, *Biochemistry*, **13**, 3816; 1974). In all cases binding takes place at a cavity between the variable domains, but the cavity has a different size and shape in the different molecules. Amzel *et al.* describe their binding site as a shallow groove 15 Å × 6 Å × 6 Å while Segal *et al.* find a large wedge-shaped cavity 12 Å × 15 Å × 20 Å and Edmundson a conical cavity 10 Å in diameter leading to a deep pocket. The groove or cavity is lined mainly or exclusively with residues from the hypervariable regions of the sequence, which in the structure cluster to form the surface of the binding pocket. Segal *et al.* argue from their results that amino acid substitutions, deletions and additions in these regions could induce such differences in the overall shape, size and general chemical nature of the antigen binding sites that the hypervariability itself could be sufficient structural explanation for antibody diversity. On the other hand neither group working on the Fab fragments has observed any significant conformational change on hapten binding of the kind that might be expected to provide a structural basis for complement fixation or B-cell activation reactions (both functions of the Fc fragment). Nevertheless, Poljak *et al.* do not rule out such a possibility and point to the asymmetric orientations of the switch regions as possible loci of structural alteration, which perhaps takes place when the true antigenic determinant rather than a simple hapten is bound.

THE continued existence of the apparently unstable West Antarctic Ice Sheet and Ross Ice Shelf is a major geophysical puzzle that only recently has been recognised. In this issue of *Nature* (page 168) Dr Gordon Robin presents new field data obtained on the Ross Ice Shelf and the very fast moving ice streams that drain the West Antarctic Ice Sheet. These field data are essential to the future solution of the puzzle.

The West Antarctic Ice Sheet forms one continuous body of ice with the Ross Ice Shelf. By definition the ice sheet becomes the ice shelf where the ice loses contact with bedrock and floats in sea water. The base of the West Antarctic Ice Sheet over much of its area is at the startling depth of 0.5 to 1 km below sea level. Moreover the bed would remain below sea level over a large area even if all the ice were removed and isostatic rebound took place. Only the great weight of the ice sheet keeps the ice pressed against bedrock. But at the edge of the ice sheet the ice thickness is insufficient to keep the ice mass grounded and here the floating ice shelf is formed. The ice thickness at the junction of the ice sheet and the ice shelf is greater than that of an equilibrium ice shelf that is nourished only by the snow that falls on it. Thus the Ross Ice Shelf becomes thinner the nearer it is to its seaward edge.

Stability of Antarctic ice

from J. Weertman

It is natural to suspect that an ice sheet whose base is so far below sea level might be unstable. Hughes (*J. geophys. Res.*, **78**, 7884; 1973) has suggested that the West Antarctic Ice Sheet is disintegrating. He thinks the junction of this ice sheet with the Ross Ice Shelf is retreating at present at a rate of about 70 m yr^{-1} . This rate of destruction of the ice sheet would raise the sea level by about 0.5 mm yr^{-1} .

On the basis of a simple-minded, two dimensional glacier mechanics analysis I found, essentially, that there is a 50-50 chance that the West Antarctic Ice Sheet is indeed disintegrating (*J. Glaciol.*, **13**, 3; 1974). A more accurate answer to the question of whether it is disintegrating and if so, how fast should be obtainable from a three dimensional glacier mechanics analysis carried out with the aid of computer calculations. Such an analysis requires the input of extensive field data from the West Antarctic Ice Sheet that have yet to be gathered as well as the field data that Robin has obtained for the Ross Ice Shelf. The question can, of course, be

answered with field observations. But it may be very difficult to determine where the Ross Ice Shelf joins the West Antarctic Ice Sheet if, as seems to be the case, there is no sharp line of demarcation. There seems to be a broad zone over which the ice alternately floats and is grounded. Furthermore, if the rate of retreat is of the order of Hughes's estimate, field measurements made over many decades would be required to obtain a reliable answer.

Robin's data on the ice streams may help solve another problem that could be related to the stability of the West Antarctic Ice Sheet and to which not even a tentative solution has been proposed in the literature: namely, why fast moving ice streams form near the edge of this ice sheet. Almost all of the ice that flows from the West Antarctic Ice Sheet moves through such streams which could be formed either in deep subglacial channels or because of an instability in the ice flow. There seems in fact to be no good field evidence that the ice streams are confined in deep channels in the bedrock. If the streams do not correspond to channels in bedrock their widths will be unstable and the West Antarctic Ice Sheet might surge by a rapid increase in the width of an ice stream. One theory of the ice ages is based on postulated surges of the Antarctic Ice Sheet.

Structure of a structural protein

from D. L. D. Caspar

PROTEINS which build viruses, muscle, membranes and other cellular assemblies stand in a separate class from the soluble proteins of protoplasm and extracellular fluids. D. A. Marvin and his colleagues at Yale have now reported the construction of the first realistic molecular model of an assembly of a structural protein. The structure is a slender bacterial virus called Pf 1 which now becomes a robust paradigm for essential macromolecular assemblies in cells and tissue.

Marvin's quest for the structure of the filamentous bacterial viruses started a dozen years ago when these μm long, 60 Å diameter particles seemed a simple model system for defining the minimal properties of a self-assembling virus. His first X-ray patterns from fibres of the fd strain showed key features characteristic of α -helical fibrous proteins: strong diffraction near the equator at ~ 10 Å spacing and near the meridian on a ~ 5 Å layer line. Spectroscopic measurements indicated that the coat protein might be all α -helical in the intact particle, and the sequence of

the 50 residue fd subunit suggested which end would interact with the DNA core. Possible α -helical models for the subunit were built long before there was any clear idea of how to pack them in the virus particle. The problem was that the symmetry of the subunit packing was hidden in the X-ray pattern. Four other strains of this virus isolated in North America and Europe gave similarly inscrutable X-ray patterns. Attempts to figure out the coat protein design from misleading electron micrographs led to fanciful two-stranded models. Marvin, Wiseman and Wachtel (*J. molec. Biol.*, **82**, 121; 1974) solved the packing problem by looking at the structure of two more filamentous viruses: Pf 1 isolated in Japan and Xf from China. These oriental strains gave readily scrutable X-ray patterns since they have regular helical symmetry (4.4 units per turn of 15 Å pitch). Moreover, the occidental strains could be seen by comparison to have similar underlying symmetry which was obscured by quasi-equivalent bonding of the subunits in a periodically

perturbed helix.

Marvin and Wachtel (*Nature*, **253**, 19; 1975) have gone on to solve the structure of the α -helical subunit in the oriental Pf 1 strain by first tracing out its backbone. This is defined by the near equatorial diffraction in the 10 Å region of the X-ray fibre diagram and by the rules for packing α helices in coiled coils which had been formulated by Crick in 1953. The α -helix axis is coiled into a segment of a left-handed conchospiral. This is the symmetrical curve on a conical surface which describes the form of conch shells and other growing helices. Each unit in the spirally thatched virus sheath interlocks over much of its length with the fifth and ninth units along the helix of 15 Å pitch. The side-chain packing cannot be seen from the X-ray pattern alone. Marvin's approach to this problem has been to fit the amino acid sequence of Pf 1 determined in collaboration with Nakashima, Wiseman and Konigsberg (*Nature*, **253**, 68; 1975) to the constraints set by the X-ray diagram and the stereochemistry. The acidic N-

terminus of the 46 residue molecule is placed outside and the basic groups near the C-terminus are arranged inside to neutralise the nucleic acid core. Following criteria worked out by Smillie and his colleagues (Hodges *et al.*, *Cold Spring Harb. Symp. quant. Biol.*, **37**, 299; 1972) for "knobs-into-holes" interlocking of the tropomyosin side chains in a coiled-coil, the Pf 1 subunit can be arranged so that most of the hydrophobic residues make favourable van der Waals contacts with those on neighbouring α helices. It looks as if this ingenious model building from sequence data refined against a low-resolution X-ray fibre pattern may lead to as clear a picture of the atomic structure of this virus as protein crystallographers now produce by automated analysis of high resolution diffraction data from isomorphous crystals.

Structural molecular biology started with model building. Pauling and Corey discovered the α helix in 1951 by folding a stereochemically correct polypeptide chain to optimise the interactions between turns. Crick looked at the interactions between chains and constructed his models for α -helical coiled coils as well as his half of the double helix in 1953. Models of these helical molecules could be built and refined since the backbone folding was seen directly in the X-ray fibre diagrams. The chain folding in globular proteins is not, however, apparent from inspection of the forest of spots in single crystal diffraction patterns. Another breakthrough in 1953 was Perutz's introduction of the isomorphous replacement method which led the way for development of protein crystallography from what he called (in a different context) "the ingenious puzzle-solving of the early pioneers to the almost blindfold automation of the present day". The structures of many soluble globular proteins which yield excellent crystals have now been solved to atomic resolution by this technique. Crystallographic scrutiny has not been comparably successful with structural proteins since good crystals with small unit cells are uncommon—perhaps because these proteins are designed to aggregate in their own way. Isometric virus particles, enzyme complexes and other globular protein assemblies have been crystallised and these structures may be solved by crystallographic methods in spite of the very large unit cells. Structural proteins with recognisable backbones—such as tropomyosin filaments which give relatively poor crystals—will be solved like the filamentous phage by refining a molecular model against low resolution X-ray data.

The structure of a virus or a cellular assembly embodies the rules for its formation. The filamentous bacterio-

Visions of a laser accelerator

from O. S. Heavens

A LITTLE over twenty years ago, Smith and Purcell observed the emission of light from a metallised diffraction grating when a 300 keV beam of electrons was directed along the surface in a direction perpendicular to the rulings. This phenomenon, which is referred to as the Smith-Purcell effect, is easily understood in terms of the oscillations induced in the charge carriers in the grating consequent upon the passage of electrons close to the surface. Oscillations occur, minute dipoles are created, and for the appropriate choice of electron velocity the radiation emitted will lie in the visible spectrum. The effect has attracted a modest interest and several papers have appeared giving detailed theoretical accounts of the effect, which can be understood conveniently in terms of leaky space-charge waves from periodic structure, generally considered as alternately conducting and insulating strips.

Curiously enough, the emphasis has been entirely on the radiation produced by the interaction of the electron beam with the structure. The reverse effect—in which we ask what would happen to an electron beam near a periodic structure when an electromagnetic wave passes over the grating—seems not to have been considered until recently (Mizuno, Ono and Shimoe: this issue of *Nature*, page 184). The intriguing possibility arises of using a coherent (laser) beam in such a way as to produce an accelerated beam of electrons. Accelerations of the order 1 GeV per metre seem to be feasible, coupled with the possibility of current pulses as short as 10^{-13} second, thus opening a new time domain to the experimentalist. It may be early to speculate that the typical large, expensive particle accelerator will become a white elephant, displaced by a laser of relatively trifling cost and an old diffraction grating. If this happens, and if laser fusion becomes a reality, optics may cease to be dismissed as an unfashionable old-hat topic neatly finished by one J. C. Maxwell!

phage is not constructed by simple self-assembly of coat protein and nucleic acid even though the design of the particle is determined by the specific bonding properties of its parts. A regulatory virus protein combines with the

viral DNA strand in the cytoplasm thereby segregating it from the replicating circular duplex and controlling its synthesis as a separate circle. The coat protein does not appear in a soluble form in the cytoplasm but is incorporated as it is made into the plasma membrane of the bacterium. Particle assembly proceeds by extrusion of the pre-packaged circular DNA molecule through the membrane bilayer where coat protein displaces regulatory protein. The assembly process is reversed in infection. Following attachment to a susceptible host by the A-protein located at one end of the virus filament, the coat protein dissolves in the membrane bilayer as the DNA and A-protein are transported into the bacterium.

In the bacterial membrane, the virus coat protein may still be folded in its α -helical conformation stabilised by the oily environment; and it may span the bilayer with its positive and negative ends on opposite sides. This picture is reminiscent of many speculative models for membrane proteins—and one recent experimental one. Richard Henderson at the MRC laboratory in Cambridge has recently seen by X-ray diffraction that the purple membrane protein of *Halobacterium halobium* is folded into α -helical segments directed approximately normal to the plane of the bilayer and these segments appear to be packed together with coiled-coil interlocking (*J. molec. Biol.*, in the press).

The filamentous bacteriophage in its different stages of development may be as useful for understanding membrane differentiation as it will be for comprehending fibrous protein organisation, regulation of DNA synthesis and control of virus assembly. Marvin has revealed this structure as a microcosm in which these basic problems in structural biology can be critically explored.

Molecular weight distribution in polymers

from Paul Calvert

GEL permeation chromatography (GPC) is the only satisfactory method of studying molecular weight distribution in synthetic polymers. Recent improvements in column packing materials and in molecular weight sensitive detectors should allow the development of this technique from a slow, poorly calibrated measurement into a rapid, accurate one.

Synthetic polymers owe their utility to their high molecular weight, and average molecular weight is a much measured characteristic. But some important

properties are particularly sensitive to the molecular weight distribution as well as to the average: for example a small fraction of very long molecules will markedly increase melt viscosity and a few short molecules can disproportionately reduce the breaking strength.

Separation in GPC is due to differential exclusion of the randomly coiled macromolecules from pores in the particles of the column packing. Larger molecules are excluded from more pores and flow faster through the column. In operation the technique is clumsy. High temperatures are needed to keep many polymers in solution and this temperature must be closely controlled along 3–4 foot columns. High pumping pressures are needed but pressure variations must be minimised. The column packing may be cross-linked porous polystyrene particles, which are difficult to pack and use, or porous glass particles, but adsorption effects make such glass packings unsuitable for polar polymers. The eluting polymer is usually detected by differential refractive index measurement. A typical run takes 2–3 hours and a change of the solvent system is a major operation. Ideally, calibration would be performed using narrow molecular weight distribution polymers under the same conditions as the subsequent runs, but only for polystyrene are such fractions readily available. This problem is avoided by use of a universal calibration curve based on the assumption of separation by molecular volume and on known viscosity–molecular weight relationships for the polymer under run conditions. This calibration has often been questioned—for instance Ostocka and Hellman in *J. Polymer Sci. (letters)* (12, 331; 1974), show that it is successful for polystyrene on glass bead columns in a range of solvents but not on polystyrene-bead columns where the pore size is probably also a function of solvent.

Thus GPC is at present suitable for application where a large number of similar samples are to be measured but lacks the flexibility or accuracy necessary for research applications and is too slow for on-line process control.

Two papers by Ouano and Ouano and Kaye describe progress in the development of a molecular weight, continuous flow detector which would eliminate the calibration problem. The first (*J. Polymer Sci. (A-1)*, 10, 2169; 1972) described the construction of a viscosity detector which measures the pressure drop in a capillary. Molecular weight could be determined to within 10% in a 10 μ l cell at concentrations of less than 10^{-1} g l $^{-1}$ and flow rates of 1 ml min $^{-1}$. The main drawback is the sensitivity to pump pulses.

A recent paper (*J. Polymer Sci.*

(Chemistry), 12, 1151; 1974) describes a low angle laser light scattering detector using a 6 μ l cell which will work at concentrations of 10^{-2} g l $^{-1}$, for molecular weights from 10^2 to 10^6 . Between the column and the cell a filter is necessary to remove fragments of column packing. The volume of the filter and its connecting tubing significantly reduces the resolution of the present system.

High speed GPC is a second recent development which is being studied in several laboratories. Unger *et al.* (*J. Chromat.*, 99, 435; 1974) describe a system using polystyrene gel particles in which a 2 foot long column of 5 μ m particles could separate a polystyrene sample in 10 min, at a flow rate of 2.5 ml min $^{-1}$. Ultimately high speed must be at the cost of resolution but the use of small particles offsets this.

Radioactive tracers in the atmosphere

from D. H. Peirson

THE use of fission product pairs with similar half-lives as meteorological tracers is described in a recent paper (Holloway *et al.*, *Journal of Geophysical Research*, 79, 4453; 1974). This is a recent example of the exploitation of radioactive tracers in the atmosphere, a technique that has developed considerably during the last twenty years.

The impetus came during the 1950s when it became necessary to determine the distribution of nuclear weapon debris by taking samples of airborne dust or rainwater and to attempt a quantitative estimate of the radioactivity in order to assess the radiological hazard. At first it was possible only to measure the gross radioactivity of the sample and then make corrections for decay, according to a crude assumption that the beta-radioactivity of the fission product mixture varied more or less inversely with time. Soon however the full sensitivity and resolution of the radioactive tracer method became available when samples of airborne dust and rainwater could be analysed for specific radionuclides.

The earliest and probably most rewarding achievement of this technique was the confirmation of the seasonal reinforcement of the troposphere in the early summer of each year by air from the stratosphere. The variation of the concentration of strontium-90 (half-life 28 year) in rainwater, and subsequently caesium-137 (30 year) in air, gave ample demonstration of the effect which had been previously indicated by the behaviour of ozone and water vapour in the atmosphere. At the same time it was possible to show that the radioactivity transferred from the stratosphere appeared in the troposphere with a maximum in mid-latitudes and minima at pole and equator.

The next advance in exploitation of the technique was to extract qualitative as well as quantitative information from these observations by measuring the radioactivity of

pairs of fission products. Thus the ratio of strontium-89 (50 day) to strontium-90, of cerium-144 (285 day) to caesium-137 and of various other combinations could be used to estimate the date of the fission explosion, or if the date was known then these ratios could indicate the proportion of nuclear debris released by the latest explosion against a background from earlier events.

Because the bulk of fission radioactivity has been inserted into the stratosphere it is possible to determine, by observations of these radioactive tracers, the delay of stratospheric residence time before the material returned to the surface of the Earth. The massive explosion series of 1961–62 were followed by several quiet years when it was possible to determine unambiguously a mean residence time in the (lower) stratosphere of about 16 months by measuring the decrement of the long-lived fission products strontium-90 and caesium-137. It was also possible to show, during this period when the northern hemisphere contained so much more fission radioactivity than the southern, that the mean residence time against interhemispheric transfer was between three and five years.

Meanwhile the radioactive tracer method has been used with natural radioactivity, derived both from the surface of the Earth and from cosmic radiation. Radon-222, emanating from terrestrial radium, has a succession of radioactive descendants, the longest-lived of which are lead-210 (22 year) and polonium-210 (140 day). These two tracers have been used, separately or relatively, as an index of the residence time in the troposphere, the resuspension of dust and the prevalence of continental or oceanic winds. A number of radioactive species are produced by the interaction of cosmic radiation with atmospheric nuclei. Of these, for example, the ratio of beryllium-7 (54 day) to phosphorus-32 (14 day) has been used to indicate vertical movements in the atmosphere.

These run times make possible process control using GPC.

The availability of rapid, accurate GPC would be a great boost to polymer research, in studies of both polymerisation and polymer properties. Many effects which are attributed to changes in average molecular weight may turn out to be more directly related to changes in the distribution. In principle, these techniques would also extend the ability of porous gel (Sephadex) columns to separate complete mixtures of proteins; it will be interesting to see if they are taken up.

Aerosol and climate: hotter or cooler?

by John Gribbin

THE puzzle of whether an increase in dust in the atmosphere leads to global cooling or global warming is given a further airing in a recent issue of *Science* (186, 827; 1974). According to some authorities this is a problem of extreme urgency, but the one sure conclusion from the latest results is that it cannot be solved by simple approximations.

Just such a naive estimate led to the original fears that man-made aerosols (that is, small dust particles, not the gases used as propellants in so-called 'aerosol' sprays) might be blocking out enough of the Sun's heat to produce a significant cooling of the Earth, perhaps leading to a new ice age. Volcanic dust had already been suggested to contribute to the onset of natural ice ages, and experiencing a hazy day will suggest to most people that dust in the air reduces the temperature. Although such dust must prevent some of the incident radiation reaching the surface, however, the question remains of how much radiation is absorbed by the dust, and to what extent the dust prevents radiation from the Earth's surface escaping into space. These are non-trivial points, as calculations by Weare, Temkin and Snell emphasise.

In simple terms, the problem

becomes one of whether the 'grey' particles of dust are distributed above a strongly reflecting 'white' ground surface, or above an absorbing 'black' surface. Earlier in 1974 Chylek and Coakley reported calculations in which an analytical solution of the radiative transfer equation produces a guide to whether aerosol particles will lead to heating or cooling for any given albedo of the underlying surface. The critical ratio is that of the absorption cross section $(1-\omega)$ to the average back-scattering cross section $(\omega\beta)$ for the aerosol particles. When this exceeds a certain critical value, heating occurs; when it is less than that value there is cooling. And this critical value is related to the albedo of the underlying system (a) by $(1-\omega)/\omega\beta = (1-a)^2/2a$, where ω is the particle single scattering albedo (*Science*, 183, 75; 1974). This confirms that the same type of aerosol will have different effects over surfaces with different albedos and seemed to provide a guide to how these effects would vary. But the further study by Weare *et al.* now shows the importance of at least one effect neglected in the earlier calculations.

This is the question of just where in the atmosphere the aerosol layer produces its greatest effect. The relation between aerosol properties and underlying albedo found by Chylek and Coakley is restricted to the situation when the aerosol layer is distributed above the Earth-atmosphere system—and that is clearly a less than realistic situation. Numerical calculations for the cases when the aerosol layer is above or below the cloud layer, and for a "well mixed" model, show, not surprisingly perhaps, that "at any given preexisting Earth-atmosphere albedo, in order for added aerosol to result in heating, the critical absorption-to-backscatter ratio for the cloud is least when the aerosol is distributed above the cloud". For an annual global average albedo of 0.29, the critical ratio is 0.9 for an aerosol layer above the cloud, 1.3 in the well mixed model, and 3.2 for an aerosol layer between

the cloud and the Earth's surface.

But even these calculations contain sweeping generalisations which require that they be used only with extreme caution in the real world. The "global average" approximation is itself an imperfect guide to reality: the calculations assume diffuse thin cloud rather than a 'lumpy' distribution of cloud cover; and aerosol particles from man's activities do not, by any means, cover the entire globe. The important conclusion from this work is that the effect of added aerosol on the radiation balance of the Earth does indeed depend both on the intrinsic optical properties of the aerosol particles and on their distribution through the atmosphere, as well as on the reflective properties of the underlying surface.

The situation also has an analogy in the problem of how aerosol particles might affect cloud cover. Acting as 'seeds' for droplet formation, they might increase cloud cover, increase the Earth's reflectivity, and produce a global cooling. On the other hand, if the particles simply attach themselves to existing droplets then 'dirty' clouds are produced, which could absorb more radiation and produce a global warming. It seems foolish for mankind to tamper with such a complex and ill understood system, and careless pollution of the atmosphere is unlikely to be a good thing. But we are still far from being able to say with confidence whether or not these polluting activities are hurrying the onset of the coming ice age.

Ocean-continent comparisons

from Peter J. Smith

THE idea has spread rapidly in the Earth sciences that some segments of continent are not truly continental at all, but are of oceanic origin. One of the best known examples of supposedly 'oceanic' continent is that of ophiolite sequences which are thought to be pieces of ancient ocean floor raised to the surface and sandwiched into continental material during the collision of tectonic plates. The importance of confirming such translocations can hardly be overemphasised, not only because of the plate tectonic implications but because it would mean that some oceanic rocks could be studied without the problems of accessibility posed by a thick cover of water. So although the evidence currently available is far from negligible, it is desirable that as many more ways as possible should be found to distinguish between oceanic and continental material. It is thus unfortunate that two potential geochemical differentiators have now proved to be unsuitable.

ANOTHER recent calculation of the effect of an aerosol layer on the heat balance of the Earth has been reported by Reck (*Science*, 186, 1034; 1974). She has looked particularly at the question of whether or not a balance between heating and cooling effects can be achieved, and finds that this is only so for underlying surface albedos in the range 0.35 to 0.60. For albedos greater than 0.60, the net effect is always a warming; for surface albedos less than 0.35 the net effect is always a cooling. This effect seems to be independent of the height of the

aerosol layer above the surface, but too much should not be read into the detailed results. As Reck stresses, the calculations assume average aerosol properties, an average underlying surface albedo, and take no account of the abundances and optical properties of water clouds. Nevertheless, they bear out results of similar but more complex earlier calculations (R. A. Reck, *Atmos. Environ.*, 8, 823; 1974). Reck therefore concludes that this simpler model "is sufficient to determine both the sign and the fact of a finite limit to the heating effect".



A hundred years ago

At the last meeting of the Photographic Society a paper was read by Mr Hooper, "On the Origin, Aim, and Achievements of the Photographic Society, with suggestions as to its future development." The suggestions were, the necessity of obtaining a Royal Charter, the Society's claim upon the Government for a money grant and suitable premises, and the necessity of forming committees for scientific investigation. In the subsequent discussion, the general opinion was that there was little hope of obtaining the proposed Charter, and that it was a mistake to speak of photography as a science. "Science," one speaker said, "had done a great deal more for photography than photography had done for science."

At the meeting of Convocation of the London University on Tuesday, the motion brought forward by Mr A. P. Hensman "That, in the opinion of Convocation, it is desirable that women should be permitted to take degrees in Arts in this University," was, after some discussion, withdrawn.

from *Nature*, 11, 236, January 21, 1875

The first such 'failure' arises incidentally from a study carried out by Schwarzer and Rogers (*Earth planet. Sci. Lett.*, 23, 286; 1974). During their investigations of the geochemistry of flow and pyroclastic rocks in Texas, Schwarzer and Rogers decided to compare the major element chemistry of all the alkali olivine basalts they could find described in the literature. The data were subdivided into continental, oceanic and island-arc sets, according to the inferred crustal environment at the time the basalts were erupted, and plotted together on $\text{Na}_2\text{O} + \text{K}_2\text{O}/\text{SiO}_2$ and AMF diagrams. Neither the major element compositions nor the differentiation trends of the alkali olivine basalts varied significantly from environment to environment.

Schwarzer and Rogers conclude first, that alkali olivine basalt is a primary magma type generated worldwide; second, that the magma is generated at sufficient depth in the upper mantle that the crustal environment above (continent, ocean or island arc) has no influence on major element chemistry; and third, that the major element chemistry of the alkali olivine basalts that reach the surface is likewise unaffected by passage through crustal material. It is thus clear that the major element chemistry of alkali olivine basalts could not be used to differentiate ocean and continent.

Mitchell and Aumento (*J. geophys. Res.*, 71, 5529; 1974), unlike Schwarzer and Rogers, were explicitly seeking an ocean-continent differentiator (for ultramafic rocks)—and with some hope of success. Previous studies had suggested that the uranium content of primary minerals in oceanic ultramafic rocks may be quite different from that of the same minerals in continental ultramafic xenoliths. Aumento and Hyndman (*Earth planet. Sci. Lett.*, 12, 373; 1971) found that the orthopyroxene in mid-Atlantic ridge ultramafics contains 1 p.p.m. of uranium on average, whereas clinopyroxene and fresh olivine poikilitically enclosed in chrome spinels contain 0.2 and 0.03 p.p.m. uranium, respectively. Kleeman *et al.* (*Earth planet. Sci. Lett.*, 5, 449; 1969), on the other hand, found that in the ultramafic xenoliths from the newer volcanics of western Victoria, most of the uranium is in the clinopyroxenes (0.22 p.p.m.) and the accessory mineral apatite, whereas orthopyroxene, olivine and spinels contain very little (less than 0.0049 p.p.m.).

It is now clear that these differences, though real, are misleading. Mitchell and Aumento find that average uranium concentrations in orthopyroxenes in ultramafics from ophiolite sequences range from 0.02 to 0.072 p.p.m. Not only are these values much lower than those from mid-Atlantic ridge orthopyroxenes (0.75–1.20 p.p.m.), they are significantly lower than those in orthopyroxenes from (continental) kimberlites (0.11–0.88). On the other hand, they are higher than the values for (continental) Australian lherzolite (0.002). And although the uranium content of the orthopyroxenes in the mid-Atlantic ridge rocks is high, fresh orthopyroxenes in other oceanic ultramafics have very low uranium concentrations. Similar variations occur in the clinopyroxenes. Thus, for example, the uranium content of clinopyroxenes in ophiolites are comparable to those in coexisting orthopyroxenes, but much lower than those in clinopyroxenes in ultramafic rocks from either the mid-Atlantic ridge or cratonic continental environments.

Mitchell and Aumento go on to describe even more confusing variations; but enough has been said here to show that a simple distinction between oceanic and continental rocks on the basis of uranium content is out of the question for the time being. Part of the problem seems to be that considerable uranium enrichment takes place during processes of alteration. So although orthopyroxene in oceanic ultramafic rocks is the mineral most resistant to serpentinisation, and thus whose uranium content is most likely to resemble that of original fresh rock, some alteration may nevertheless have

taken place. For example, the mid-Atlantic rocks used by Mitchell and Aumento were from 45° N to 52° N and highly altered; and it is probable that the alteration has reached the orthopyroxene on a submicroscopic scale. Other mid-Atlantic (and mid-Indian Ocean) orthopyroxenes, reported by Seitz and Hart (*Earth planet. Sci. Lett.*, 21, 97; 1973), do have uranium concentrations which are low and comparable to the corresponding values from ophiolites. It is possible, therefore, that more use may be made of oceanic uranium concentrations when the alteration processes are more fully understood.

The uranium variations within continental ultramafics may also be due in part to differential alteration, although some may arise from different modes of formation. Nishimura (*Chem. Geol.*, 10, 211; 1972), for example, has suggested that uranium content may vary with depth of origin. Again, a much more thorough knowledge of the processes involved will be required before uranium content can even be considered as an ocean-continent differentiator.

Theory of the nuclear optical potential

from P. E. Hodgson

ONE of the most basic interactions in nuclear physics is that between a nucleon and a nucleus. If we know this interaction we can calculate the cross section for elastic scattering and also the distortions of the nucleon waves by the nucleus which are essential for a thorough understanding of the reactions that can take place.

This interaction is really the sum of all the individual interactions between the incident nucleon and the nucleons of the target nucleus, modified by the correlation effects due to their proximity. As it is extremely complicated, many studies have been made to see how accurate it is to replace it by a simple one-body potential that takes no explicit account of the structure of the target nucleus. This potential has a radial shape very similar to that of the nucleus itself and has a real part and an imaginary part of which the former may be thought of as refracting the incident nucleons and the latter as absorbing them: the absorption takes account of all inelastic scattering and reaction processes that remove flux from the elastic channel. The one-body potential is called the optical potential because of the similarity between the scattering and absorption of nucleons by nuclei and the scattering and absorption of light by a refracting and absorbing medium: the former can be described by a complex potential and the latter by a complex refractive index.

This simple idea has proved astonishingly successful and it is now possible to account for the elastic scattering cross sections for many nuclei at many energies by suitably choosing the parameters of the potentials. It has been developed by the addition of a spin-orbit term, which allows the polarisation of the scattered nucleons to be calculated as well. The same potentials have been extensively used to generate the distorted waves needed in nuclear reaction calculations.

One of the main difficulties with these optical potentials is that they are frequently ambiguous; that is, it is possible to find several different potentials that give equally good fits to the experimental data. These ambiguities are much reduced as the precision of the data is improved, but some of them still remain.

Thus the purely phenomenological approach to the determination of the parameters of the optical potential, in which its parameters are systematically adjusted to optimise the fit to the experimental data, is inadequate. It needs to be supplemented by a more basic calculation of the potential from the constituent nucleon-nucleon interactions. Although this is very difficult and complicated, and many approximations have to be made in order to make the computations tractable, it is nevertheless able to give valuable information on the optical potential that substantially reduces the objectionable ambiguities.

The simplest of these calculations gives the real part of the optical potential as the folding of the nucleon-nucleon interaction $v(|\mathbf{r}-\mathbf{r}'|)$ with the nuclear density $\rho(\mathbf{r}')$ where $\mathbf{r}$ is the vector to the incident nucleon and $\mathbf{r}'$ that to a nucleon in the target:

$$U(r) = \int v(|\mathbf{r}-\mathbf{r}'|)\rho(\mathbf{r}')d\mathbf{r}'$$

Many calculations using this approach, following the work of Greenless, Pyle and Tang (*Phys. Rev.*, **171**, 1115; 1968) have proved very successful.

The imaginary part of the potential is more difficult to calculate because in principle one should take account of all possible reaction processes, but some progress has been made by considering its strength as simply given by

$$W = \frac{1}{2}\hbar v\bar{\sigma}$$

where v is the velocity of the nucleon, ρ the nuclear density and $\bar{\sigma}$ the mean collision cross section. This relation follows from the semi-classical theory of the optical potential.

The success of these calculations has stimulated many more detailed theories of the optical potential. Among these some very successful calculations have recently been completed by Jenkenne, Lejeune and Mahaux of the University of Liège (*Physical Review*, **C10**, 1391; 1974). In this work, the calculations were first of

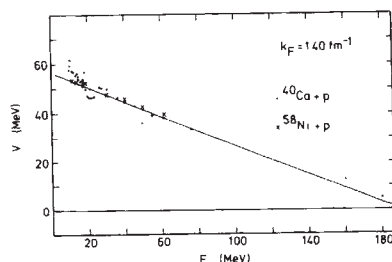


Fig. 1 Calculated strength of the real part of the optical potential as a function of incident nucleon energy compared with phenomenological potentials obtained from the cross sections for the elastic scattering of protons by ^{40}Ca and ^{58}Ni .

all made for infinite nuclear matter, and then the results were applied to finite nuclei using the local energy approximation. This means that we solve the problem for infinite nuclear matter as a function of its density, and then apply the results to nuclei using their known density distributions. Thus the potential at a point of density ρ is assumed to be that given by calculations of the properties of infinite nuclear matter of density ρ . This approximation is justifiable if the nuclear density changes slowly over distances of the order of the mean free path of nucleons in nuclei, which is certainly true in the nuclear interior but is more questionable in the nuclear surface. The reason for making the first calculations in infinite nuclear matter is because there the nucleon wavefunctions are plane waves, which naturally simplifies the calculations.

The nuclear matter calculations were made by solving the Bethe-Goldstone equation, which describes the interaction of two nucleons inside the nucleus. To do this calculation we need to know the interaction between two nucleons in free space, and this is known quite well from many phenomenological analyses of the scattering of protons by protons and by neutrons at many energies. Of several forms now available the Reid potential was chosen as it has already been successfully used in a wide range of nuclear matter calculations. Inside the nucleus the nucleon-nucleon scattering is modified by the presence of the other nucleons and in particular many interactions are forbidden by the Pauli exclusion principle as

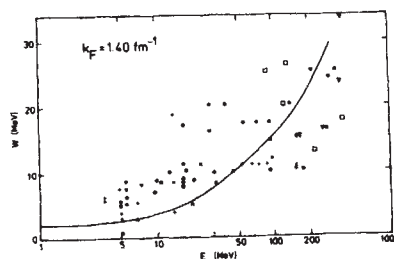


Fig. 2 Calculated strength of the imaginary part of the optical potential as a function of incident nucleon energy compared with a number of phenomenological values.

they proceed to states that are already occupied. This effect, incidentally, ensures that the mean free path of nucleons in nuclear matter is long enough for the shell model to be a valid description.

The solution of the Bethe-Goldstone equation and the extraction of the optical potential requires lengthy calculations, and some of the results are shown in the figures. Fig. 1 shows the strength of the real part of the potential as a function of energy calculated for a Fermi momentum in nuclear matter of 1.40 fm^{-1} and taking account of the various terms in the mathematical expression for the potential. It is compared with phenomenological potentials obtained by analysing the elastic scattering of protons by ^{40}Ca and by ^{58}Ni . Up to about 100 MeV the radial dependence of the real potentials obtained in these calculations is very similar to the Saxon-Woods form

$$f(r) = [1 + \exp\{(r-R)/a\}]^{-1}$$

used in most phenomenological analyses, but that at higher energies shows a pronounced surface peaking. Already some phenomenological indications favouring such a form have been found, and it will be important to take this into account in future analyses of the data.

The calculated energy variation of the strength of the imaginary part of the optical potential is compared with some phenomenological determinations in Fig. 2. Its radial dependence changes from a predominantly surface-peaked form at the lower energies to a predominantly volume form at higher energies, and this is just the same behaviour as the phenomenological imaginary potentials. In phenomenological analyses there is much ambiguity between the amounts of volume and surface components, so that it is difficult to determine them individually. It will be a great advantage to have available some reliable theoretical guidance for the relative contributions of volume and surface absorption.

This work is in process of extension by taking into account some of the higher order terms in the solution of the Bethe-Goldstone equation, which should give improved optical potentials. Already the results obtained are most encouraging, in that they reproduce many of the features of the optical potentials already known from phenomenological analyses. As confidence in the reliability of such calculations grows, they will play an increasingly important part in guiding optical model analyses of elastic scattering cross sections. They should provide the essential behaviour of the form factors, leaving only the strength to be improved over the calculated values by optimisation of the fit to the experimental data. This should provide more reliable potentials that will improve the accuracy of the information on nuclear structure obtained from analyses of nuclear reactions.

review article

Chromosome imprinting and the mammalian X chromosome

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Chromosome imprinting is the process by which one of two genetically homologous chromosomes is predetermined to function differently from the other at a subsequent stage in development. In the coccid insects, imprinting occurs in the egg, at the time of fertilisation; it probably occurs at the same time and site in mammals, and possibly also in Sciara.

DURING development, a chromosome from the father may function quite differently from its maternal homologue, a fact discovered in the 1920s by Metz¹ in *Sciara*, a dipteran insect. According to his colleagues (C. Stern, personal communication), Metz often asked, "How do the chromosomes know which parent they come from?" Almost fifty years later, this question remains unanswered. Since then, the *Sciara* system has been explored in much greater detail, and systems of differential regulation of homologous chromosomes have been discovered in two additional groups of organisms. In the 1930s, the Schraders² analysed the chromosome system of the mealybugs and other coccid insects. In 1961, Lyon³ described the system in eutherian mammals; ten years later, a group of Australian workers^{4,5} reported a somewhat different situation in marsupials. It is the purpose of this account to outline similarities among these three systems and to show that analyses of such types of chromosome behaviour provide important clues to the nature of genetic regulation during early development.

Chromosome imprinting

Crouse⁶ introduced the term imprinting to indicate the process by which differential behaviour of the members of a pair of homologous chromosomes is predetermined several to many cell generations before the stage in development at which resulting behavioural differences become obvious. She provided an operational definition of imprinting, based on her work with *Sciara*, which should suffice until the imprinting mechanisms are more clearly understood. Confusion from use of the same term in behavioural sciences can be avoided in genetics by use of the complete term, chromosome imprinting.

It is not known how, in molecular terms, the differential chromosome behaviour originates, or, having arisen, is maintained in subsequent cell lineages; but some progress is being made in showing when and where the chromosomes are subject to imprinting. In her original definition, Crouse⁶ assumed that both paternal and maternal chromosomes carry an imprint. Imprinting of only one of two homologous chromosomes is, however, sufficient to account for differential behaviour and this simpler assumption will be followed here.

In the following sections, the systems involving differential behaviour of homologues will first be reviewed as a basis for understanding the evidence available from each on the process of imprinting.

Significant features of the three imprinting systems are compared in Table I.

The system in mealybugs

Since mealybugs and certain related coccids provide unambiguous evidence of the site and time of imprinting, this system will be reviewed first. In males, the paternal set of chromosomes becomes heterochromatic during early embryogeny and remains so in most but not all tissues during subsequent development. Meiosis is highly modified and there is no recombination. At first division, both maternal and paternal chromosomes divide equationally. At second division, the paternal, heterochromatic chromosomes are segregated from the maternal, euchromatic chromosomes. Of the four products of meiosis, only the two euchromatic derivatives form sperm; the heterochromatic derivatives degenerate *in situ*. Such heterochromatization does not occur in females^{2,7}.

The genetic inertness of the paternal set has been demonstrated by several methods, including tests with genetic markers which are expressed in and transmitted by the sons only when received from their mothers⁸. Genetic activity of the paternal set is restored, however, in those tissues in which heterochromatization is reversed during development. There is thus a strict relationship between heterochromatization and the absence of detectable gene function. Heterochromatization of the paternal set is first detectable in male embryos at about the sixth cleavage when some nuclei migrate to the periphery of the egg. This is probably the stage at which gene action begins in the mealybug embryo as the incorporation of ³H-uridine by the chromosomes cannot be demonstrated earlier⁹.

Imprinting in mealybugs

Coccid chromosomes have a diffuse kinetochore and chromosome fragments function as independent entities. After maternal irradiation, euchromatic fragments appear in male embryos; after paternal irradiation, they are heterochromatic. Thus,

imprinting itself and the process responsible for its later expression as heterochromatization are effective along the length of the chromosome¹⁰. Somewhat similar evidence has been obtained from translocated chromosomes each carrying a euchromatic and a heterochromatic segment; these chromosomes behave normally until the second meiotic division when bridging results from movement of the euchromatic and heterochromatic segments to opposite poles^{11,12}.

Evidence on the place and time in development at which imprinting occurs comes from Nur's¹³⁻¹⁵ work with parthenogenetic soft scales, a group of coccids related to the mealybug. Two distinct types of parthenogenesis were found. In one type, the second polar body substituted for the sperm to produce a diploid 'zygote'. Heterochromatization was not found in any of the four species studied. In the other type of parthenogenesis, the egg nucleus divided to produce two haploid daughter nuclei which then fused to form a diploid, homozygous 'zygote'. This type of parthenogenesis was studied in three species. In two of them, both females and males were produced parthenogenetically (type A); the males were produced toward the end of the period of egg production and apparently never functioned because there were no sperm in the numerous females examined. In the third species, functional males were produced parthenogenetically and the females arose from fusion of eggs and sperm (type B). Heterochromatization in all three species was typical. One haploid set was heterochromatized and the other remained euchromatic. In these cases, therefore, imprinting must have occurred within the egg cytoplasm and it must have affected just one of the two haploid division products of the egg nucleus, presumably during the brief period the two haploid nuclei were separated from each other. In the species in which both males and females are produced parthenogenetically, genetic factors cannot be involved because the mothers also originate by fusion of two identical haploid sister nuclei and are therefore themselves completely homozygous. Since it is very unlikely that a new mechanism would have evolved to produce the unnecessary males in the type A parthenogenetic system, the situation in the sexually reproducing species must be the same, that is, the chromosomes of the sperm must be imprinted within the egg before fusion of egg and sperm pronuclei.

All sperm of a male mealybug carry identical, maternal chromosome sets, yet both sons and daughters result. In line with the conclusions from parthenogenetic soft scales, types A and B, male embryos are produced by eggs in which paternal chromosomes are imprinted and female embryos by eggs in which they are not. Two interpretations are possible. An imprinting region could be present in some eggs, absent in others; or the region could be variable in extent, sometimes large enough to affect the nucleus in question, sometimes not. The mealybug egg is spacious enough, 0.40 mm. × 0.25 mm., to permit such internal differentiation.

When present, the imprinting region within the egg seems to be restricted. It clearly does not include the egg nucleus because the maternal chromosomes are not heterochromatized in the males of sexually reproducing species. The polar bodies also apparently escape imprinting. No males, nor any heterochromatization occurred in the four examples of parthenogenesis in the soft scales in which the second polar body substituted for the sperm. In the mealybugs, the polar bodies do not degenerate but give rise to certain polyploid tissues; heterochromatization does not occur in these complex cell lineages in either males or females⁷. Further evidence that mealybug polar bodies are not imprinted comes from crosses of heavily irradiated males and virgin females; the offspring are all gynogenetic females, and originate from one or both polar bodies. Such gynogenetic females can be triploid, diploid or mosaic, but their chromosomes are always euchromatic^{16,17}.

The system in *Sciara*

There is no differential heterochromatization in *Sciara* except briefly during development of the germ line, and the paternal chromosomes are genetically active^{1,18}, but differential behaviour involves entire chromosome sets in *Sciara* as it does in the mealybug.

The *Sciara* zygote contains two sets of autosomes and three X chromosomes, two of which are of paternal origin. Its constitution can be symbolised $A^m A^p X^m X^p X^p$, in which m and p indicate maternal and paternal origin, respectively, of a set of autosomes (A) or an X chromosome (X). During embryogenesis, one X^p is lost from the germ line of both sexes and from the female soma; both X^p chromosomes are lost from the male soma. The resultant constitutions are: female and male germ line, female soma, $X^m X^p$; male soma, X^m only. The somatic constitutions are thus the conventional AAXX (♀) and AAXO (♂) even though the germ lines of both sexes are AAXX. Meiosis is atypical only in the male; at the first meiotic division, the paternal chromosomes are eliminated. During the second division, the two sister chromatids of the maternal X move precociously to one pole. A sperm is formed from the single meiotic product which contains the precocious X chromatids (now chromosomes); the other products degenerate. Because all sperm contain two X chromosomes, all zygotes contain three.

Imprinting in *Sciara*

Understanding of the *Sciara* system is simplified by the presence of two types of females: AAXX' which usually produce only daughters, and AAXX, which usually produce only sons. This feature simplifies the question of the localisation of imprinting. A single male can inseminate females of the two types; in the one, the chromosomes of the sperm are altered; in the other, they are not. Yet all sperm carry the same set of maternal chromosomes. Since the behaviour of the sperm

Table 1 Cytogenetic characteristics of the three imprinting systems

	Mealybugs and related coccids	<i>Sciara</i>	Marsupials	Mammals
Type of centromere	Diffuse	Localised	Localised	Localised
Sex chromosomes	None	Soma: XX(♀)-XO(♂)	XX(♀)-XY(♂)	XX(♀)-XY(♂)
Differential behaviour occurs in	Male	Male	Female	Female
Chromosome imprinted	Entire paternal set	Entire paternal set but X differentially	X chromosome	Autosome*
Parental origin of imprinted chromosomes†	Paternal	Paternal	Paternal	Paternal autosome*
Heterochromatization	Yes	No	Yes	Yes (X chromosome)*
Genetic inactivation	Yes	No	Yes	Yes (X chromosome)*
Site of heterochromatization	Germ line and soma		Soma only	Soma only
Reversal of heterochromatization may occur in	Soma		Not detected	Not detected
Meiosis	Paternal set eliminated	Paternal set eliminated	Typical	Typical

*Proposed model: an autosome is primarily affected, the X chromosome secondarily; see text.

†In typical bisexual reproduction; see text for parthenogenetic examples.

nucleus is determined by the type of egg it enters, the evidence that the fate of the paternal chromosomes is determined within the egg is incontrovertible. If imprinting does not occur within the egg but rather in the body of the father, eggs of AAXX' females must be able either to erase the imprinting or fail to facilitate its expression. The kinds of parthenogenesis seen in coccids which could provide definitive evidence that the egg is the site of imprinting have not so far been observed in *Sciara*.

By appropriately manipulating the chromosomes, *Sciara* can be induced to produce exceptional offspring, sons from AAXX' mothers, and daughters from AAXX mothers^{19,20}. Since such exceptional offspring have the somatic chromosome complement expected of their sexes, it follows that sex is determined by the chromosomes. The genetic constitution of the mother determines only the chromosome eliminations which will occur and does not influence the sex of the offspring. The exceptional males arising from AAXX' mothers are, however, sterile, probably because their germ line constitution is AAXO rather than the normal AAXX²⁰.

The special behaviour of the *Sciara* X chromosome is determined by a controlling element located close to the centromere⁶. This controlling element is probably altered during the second meiotic division so that one of the then sister chromatids can later respond differently either to imprinting or to the later influences responsible for its expression.

In X-autosome translocations, the derivative carrying the X centromere conforms to the X-chromosome type of behaviour in its manoeuvres during development. It is reasonable to assume, therefore, that imprinting in *Sciara* need occur only at or near the centromeres.

The system in mammals

As is well known from confirmations of the hypothesis proposed by Lyon³, only one of the two X chromosomes is genetically active in somatic cells of female mammals. During early embryogeny of eutherian mammals, the active X is chosen at random and could be either maternal or paternal in each cell. In contrast, in marsupials it is the maternal X that is active and the paternal X is inactive or largely so^{4,5,21}. In both cases, inactivation of one X chromosome in the female serves as a dosage compensation mechanism by equating the single active X of the XX female with the single active X of the XY male. In both sexes there is the same balance between one functional X chromosome and two sets of autosomes. Selection for genes capable of functioning adequately in this situation offers a possible explanation^{22,23} for the relative evolutionary constancy of the mammalian X in regard to both size and content²⁴. The eutherian system is selectively advantageous because random inactivation confers a "mosaic heterozygosity" which is not possible in the marsupial system²⁵. Indeed, the apparently large effect of heterozygosity for human X-linked genes may result from mosaic heterozygosity²⁶.

The eutherian system can be derived from the marsupial system by a single mutational step²⁵. According to this scheme, the marsupial X carries a sensitive site and a receptor site adjacent to it. Imprinting of the sensitive site on the X chromosome brought in by the sperm leads to the inactivation of the site. The sensitive site on the X chromosome contributed by the egg is not so imprinted; it remains active and produces an 'informational entity' which attaches to the adjacent receptor site. At a later stage in embryogeny, the X without the informational entity (that from the sperm) becomes heterochromatic and genetically inactive; the X with the attached entity (that from the egg) remains euchromatic and active or potentially active.

To obtain the eutherian system, it is necessary to assume that during evolution a translocation moved the sensitive site from the X chromosome to an autosome and that the receptor site continued to remain on the X chromosome. Since the sensitive site is dissociated from the receptor site, the informational entity produced by the maternally-derived sensitive site would attach at random to the receptor site of either the paternal

or the maternal X chromosome. Translocation of the sensitive site to an autosome is thus the only step necessary to change the marsupial to the eutherian mode of regulation. As a consequence, in eutherians, the number of active X chromosomes would be the same as the number of maternal chromosome sets except when there is monosomy or trisomy for the autosome carrying the sensitive site²⁵.

Imprinting in mammals

What little evidence there is on site of imprinting in mammals comes from human ovarian teratomas^{27,28} and a human diploid/triploid mosaic^{29,30} (Fig. 1). Ovarian teratomas are usually benign tumours which resemble sexually produced embryos in that they often contain tissues such as hair and teeth. Their chromosome constitution, 46XX, is normal. No genetic markers are present in the teratomas other than those in the woman. The fact that some maternal markers are missing, however, indicates that teratomas are postmeiotic products of maternal origin. The most plausible interpretation of these data is that the abortive development culminating in a teratoma is the result of "re-entry" of the second polar body (PB2) and its subsequent fusion with the egg²⁸.

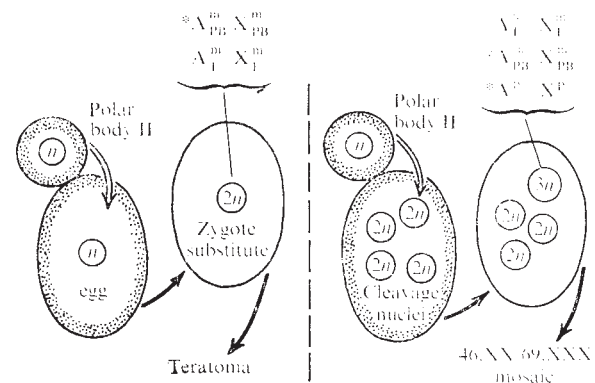


Fig. 1 Presumptive origin of human ovarian teratomas^{27,28} (left) and a human diploid/triploid mosaic female^{29,30} (right) and interpretation of their sex chromatin status²³. Stippled areas denote the regions in which imprinting would occur. A and X denote, respectively, an autosomal set and an X chromosome; superscript p indicates paternal origin and m, maternal origin; subscripts PB and E refer to polar body and egg, respectively. n , $2n$, $3n$ denote haploid, diploid and triploid nuclei, respectively. Asterisks indicate the imprinted chromosome sets. In the teratomas, the sensitive site of one of the two haploid maternal nuclei (that from polar body II) is assumed to be imprinted, resulting in one X being active and the other inactive. Similarly, in the mosaic female, imprinting of the second maternal nucleus (again from polar body II) in the triploid cells would explain the fact that, contrary to expectation (see text), two sex chromatin bodies were found in her triploid cells.

Since the teratomas have two maternal sets of autosomes, they would, on the basis of the proposed model of regulation, be expected to have both X chromosomes active; but in fact, one X chromosome was found to be inactive (sex chromatin)^{27,28}. The presence of an inactive X in these teratomas provides the most convincing evidence yet that the factors regulating X chromosome inactivation can be exclusively maternal in origin²³.

In the diploid/triploid (46XX/69XXX) mosaic^{29,30}, the extra set of chromosomes was maternal in origin, but whether it was genetically identical to the maternal set in the diploid tissues is not known. The simplest explanation for the origin of the mosaicism is, again, that the haploid PB2 re-entered and fused with one of the early cleavage nuclei (diploid) to form the triploid tissues. As in normal females, a single sex chromatin body was seen in diploid cells, but two were seen in triploid cells³⁰ although two autosomal sets of maternal origin would lead to an expectation of two active and one inactive X chromosomes²⁵.

Contrary to these expectations, two maternally derived chromosome sets were associated with an inactive X in both teratomas and in the triploid tissues of the mosaic girl. In both cases, however, one of the two maternal complements was probably provided by the second polar body. It is also significant that as in the parthenogenetic soft scales (above, types A and B), the two maternal complements were presumably separated from each other before fusion. If the assumption is made that one of the two maternal complements (polar body II) was imprinted during the period of separation, then these exceptional data would no longer be inconsistent with the model²³. If, on the other hand, the maternal contribution to the individual is in the form of a single egg nucleus, then the number of active X chromosomes is the same as the number of maternal autosomal sets—whether the egg is haploid, diploid (for example because of suppression of the second meiotic division by colchicine³¹), or aneuploid for the sex chromosomes²⁵. The simplest interpretation of the mammalian data is that the imprinting zone includes all or nearly all of the periphery of the egg²³.

In three other digynic triploid/diploid mosaics (see ref. 33), both Xs of the XXY or XXYY triploid sectors were active, thus indicating that the second maternal contribution could not have been imprinted. Differences in imprinting of the maternal genome may stem from the variable behaviour of PB2; in mice PB2 may either be extruded or retained in the egg proper to form a second female pronucleus³³. In its usual place, PB2 would be subject to imprinting and, on re-entry, would provide the stimulus necessary to initiate a teratoma²⁸. If retained within the egg proper, PB2 would be imprinted only if it came in contact with the periphery of the egg before fusing with a cleavage nucleus to initiate the triploid cell lineage. PB2 has never been observed to undergo division in mammals³³; evidence for its fusion comes solely from teratomas and mosaics of the type considered here.

It therefore seems that polar bodies of mammals, but not those of coccids, may be subject to imprinting. Furthermore, determination of sex in mealybugs is by imprinting (or the two events are at least coupled) so that capacity to imprint must be variable enough to permit production of both sexes. In mammals, on the other hand, sex is determined by the chromosomes, and X chromosome behaviour proceeds according to schedule regardless of sex of the embryo; there is a single active X in both XY and XXY males²⁵.

In conclusion, (a) the egg is the site of imprinting, almost certainly in mealybugs, probably in mammals, and possibly in *Sciara*; (b) imprinting of sperm seems to be a variable process within the eggs of mealybugs and related coccids and a consistent process within mammalian eggs; (c) if two maternal chromosome sets in mammalian eggs fuse after prior separation, one may become imprinted; and (d) in both coccids and mammals there is no necessity for assuming, as may be necessary for *Sciara*, a second level of control to explain the data.

Finally, in all three genetic systems, entire chromosomes are subject to differential regulation even though only one or a few loci may be responsible for initiating differential behaviour. Immunoglobulins represent the only other case known in which homologous genetic loci function differently within a single cell³⁴. Whether this unusual behaviour—believed to be related to the function of immunoglobulins—is also based on mechanisms analogous to imprinting is not known.

S.W.B. is supported by a grant from the US National Science Foundation and H.S.C. by an institutional grant and a grant from the World Health Organization.

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article

Ice shelves and ice flow

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New data on the Ross Ice Shelf provide an insight into the importance of the momentum with which an ice stream enters the shelf for the overall velocity field.

OF the 13,800,000 km² of ice covering Antarctica, some 12,100,000 km² lie on a rock base, while about 1,500,000 km²

are in the form of floating slabs of ice round the periphery of the continent. The largest of these is the Ross Ice Shelf. In contrast to the inland ice, which built up into a vast relatively flat dome until surface slopes and ice thickness produced sufficient gravitational forces to drive ice outwards against frictional drag at the base of the ice, the floating ice shelves rest effectively on a frictionless base. The first theoretical ideas

treated ice shelves as flat plane parallel slabs of ice spreading under their own weight^{1,2}, but observations³ soon showed that ice shelves slowly increase in thickness as one moves in from the seaward boundary, known as the ice front. This thickening was satisfactorily explained in terms of drag, mainly on the sides of the ice shelves, but also by local grounding⁴⁻⁶. No theoretical studies or relevant field observations were, however, made of the corresponding problem of faster moving streams of ice within an ice shelf. This problem will now receive considerable attention as the various studies of the Ross Ice Shelf Project (RISP) develop.

The observations

To provide the first data for planning this programme, extensive airborne soundings of the ice shelf were made between

1967 and 1972. These are presented in this paper. On the basis of: (a) these new measurements of ice thickness; (b) earlier measurements of velocity; and (c) the assumption of mass continuity, it is possible to estimate the direction and speed of ice flow over the entire shelf. These predictions will be tested by direct measurements of flow velocity as the Ross Ice Shelf Geophysical and Glaciological Survey operations are continued during the present Antarctic summer and the years following, as part of the RISP.

The new map of the thickness of the Ross Ice Shelf (Fig. 1) is based on profiling of ice thickness by radio echo sounding from aircraft. The thin, approximately straight lines on the map show aircraft flight lines along which continuous profiles of thickness are available. These total some 35,000 km of track as compared with about 3,000 km which had been

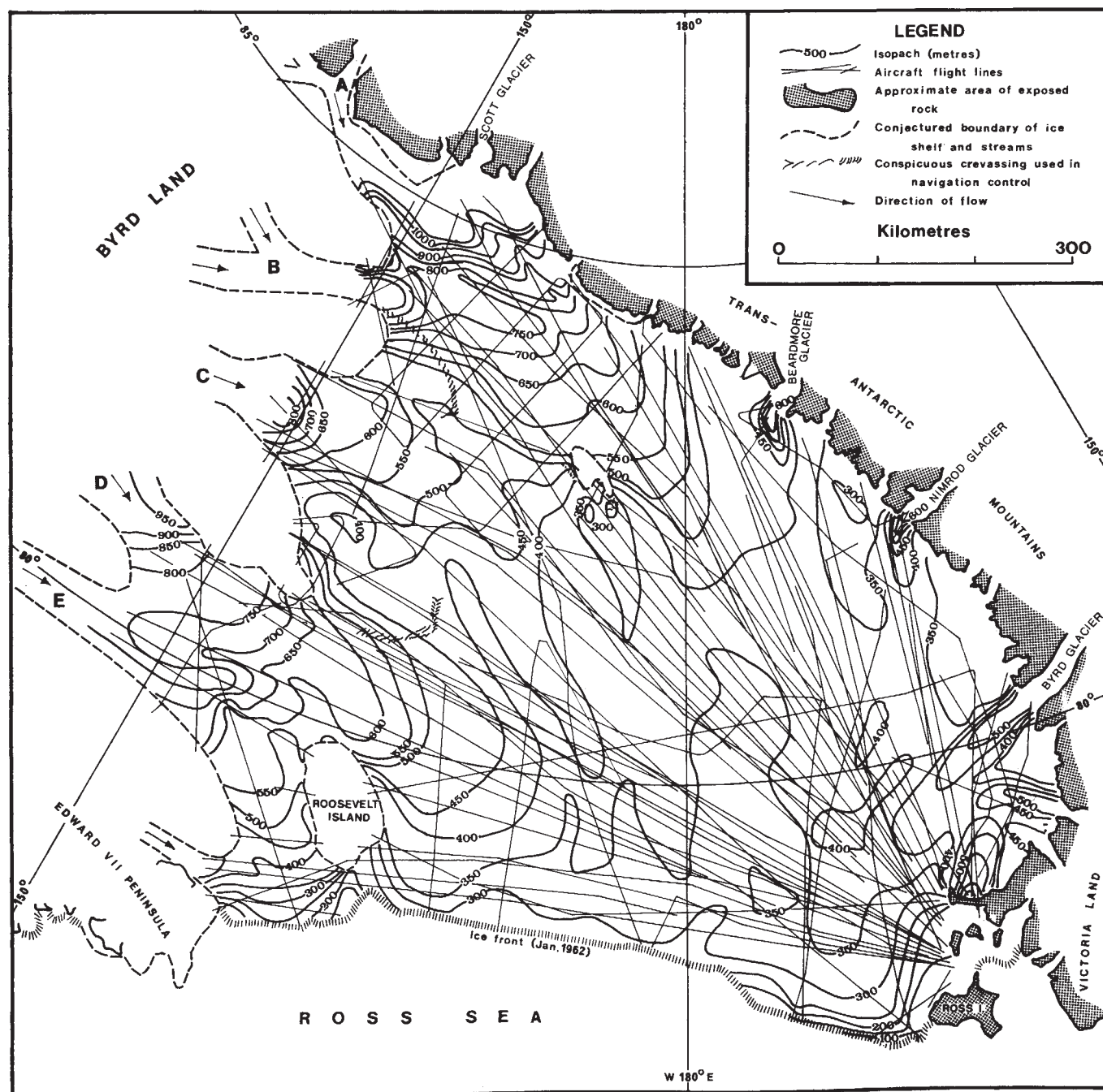


Fig. 1 Flight lines on which data were gathered.

measured previously by other methods (some 50 seismic soundings plus levelling along surface traverses). The data presented in this paper were obtained as part of the joint Scott Polar Research Institute-US National Science Foundation programme of sounding the Antarctic ice sheet from long range aircraft of the US Navy experimental squadron VXE6.

Two networks of flight lines are shown on the map because some of the flights combined logistic and research tasks. Lines which are approximately radial from the southern end of Ross Island are from flights which were made to sound more distant areas or to supply fuel to Byrd station or the South Pole; lines that cut across the radial pattern are from flights flown specifically for sounding the Ross Ice Shelf.

The importance of this new information is the clearly marked distribution of ice thickness. It can be seen, for example, that the major glaciers entering the ice shelf persist for some distance as streams of thicker ice within the shelf. The possibility of deducing patterns of ice flow from distribution of thickness offers a new tool of considerable importance in the study of the flow and deformation of ice shelves.

Accuracy of the data

Before discussing the implications of the new data, we must first consider the accuracy of measurements and the related problem of aircraft navigation. The general technique of radio echo sounding has been discussed in detail elsewhere⁷⁻¹⁰. When sounding ice shelves, as distinct from inland ice, the strength of the bottom echo is generally sufficient to permit soundings from aircraft flying at their economical cruising altitude of from 7,000 to 10,000 m, as opposed to the lower altitudes necessary for sounding of inland ice. A time delay on the oscilloscope trigger, plus an appropriate sweep speed, was used to spread the echoes from top to bottom of the ice shelf over a substantial portion of the oscilloscope screen, and hence of the recording film. Under these circumstances, errors of measurement of ice thickness are of the order of 10 m, of which 4 m are due to scaling off the film record and about 1.5% due to uncertainty over the velocity of radio waves in ice and firn.

An overall test of accuracy comes from comparison of thicknesses interpolated from Fig. 1 with those at 44 locations measured by seismic sounding¹¹. The arithmetical mean difference with regard to sign was only 4 m, the radio echo depths being the greater. The root mean square deviation between the two sets of measurements was 25 m and the maximum difference 67 m. These results indicate that the main sources of error probably come from errors of navigation and interpolation, since both are likely to produce random errors which will cancel out when averaged over many observations. The remaining difference of 4 m between the two systems is not significant.

Navigation was the chief difficulty. Most of the flight lines in Fig. 1 were flown during the 1969-70 season in a C130 (Hercules) aircraft at 7,000 to 10,000 m. Conventional navigation methods (TACAN, Sun shots, radar fixes, air photographs when within 150 m of mountains) were used. Although errors of up to 50 km may be expected, careful navigation by the aircrew, subsequent checking of interpolated (dead reckoning) positions and the relatively slow variation of wind speed and direction in the upper troposphere in summer, all served to limit errors. After some flight lines were rejected because of inconsistencies of navigation, the selected lines were plotted in a series of colours along each line, each segment of colour corresponding to a given 50 m range of depths. Then, since the remaining navigational errors were not large, a clear pattern of colour emerged on which isopachs (lines of equal thickness) could be drawn with confidence. Variations of thickness between different flight lines indicate that naviga-

tional errors were generally less than 16 km. Where there was a high density of flight lines, averaging of navigational errors should reduce errors in the position of isopachs to 10 km or less.

In 1971-72, inertial navigation (Litton 51 C) fitted to the aircraft gave a continuous record of position to an accuracy of better than 5 km for two flights that were made partly over the ice shelf. On one of these flights, four parallel lines that were flown over the south-eastern corner of the ice shelf did much to resolve the detailed pattern of thickness, and subsequently of ice flow, in this key area.

Results of four flights in 1967 by C131 J (Super Constellation) aircraft are also included. These were made at lower levels (1,000 to 2,000 m) where winds are more variable and navigational errors greater. The flight paths have been adjusted to match the thickness data from these flights to those obtained during the 1969-70 season.

Thickness pattern and streamlines of flow

Figure 1 shows the extent to which glaciers persist as streams of thicker ice within the shelf. The same effect is seen in relation to the streaming of thinner ice, especially downstream of the small ice rise around 83°S, 172°W. It is possible that the thinner stream of ice starting around 82°S, 164°W may also be caused by local grounding in this area, even though no ice rise is present. Alternatively this thinner stream may be the result of shear between ice streams moving at different speeds. Similar thinning is seen around 84.5°S, 155°W between ice streams A and B and between ice streams D and E around 80.2°S, 155°W.

In the central part of the ice shelf, where the ice shelf varies slowly between 450 and 300 m in thickness, the isopachs are less accurate, and it is more difficult to deduce streamlines. The isopachs in this area represent mean values from a number of flight lines, especially for the closed isopachs for 350 and 400 m between 170°E and 180° in which most but not all ice thicknesses exceed the indicated values. Near the ice front the effects of bottom melting appear to dominate the thickness pattern, especially in the vicinity of Ross Island, where rapid melting has been shown to take place^{11,12}.

It is clear that in order to deduce directions of ice flow, we must use these indications of streaming, rather than attempt to use mean surface slopes as is done in the study of inland ice. (In the case of floating ice shelves, surface slopes are proportional to thickness gradients.) Nevertheless, there is a general decrease of ice thickness as one moves along individual flowlines towards the ice front, and even in the few areas in which the ice thickness does increase towards the ice front, the rate of increase is slow and is probably due to new accumulation along the line of flow rather than compression of ice in this direction. Indeed there is little evidence from the map that compression along a flowline can take place, although compression occurs normal to flowlines over much of the ice shelf. This agrees with the concept that ice shelves spread out under their own weight while floating on a frictionless base^{1,2}.

The increasing thickness of ice shelves with distance from the ice front, which is explained by drag at the lateral boundaries of the shelves, has a counterpart in individual ice streams within an ice shelf. When a rapidly moving stream enters the shelf, it will be retarded by the slowly moving ice on either side, with the result that its thickness increases in the upstream direction. But in the ice to either side the opposite effect takes place, the rapidly moving ice stream drags the surrounding ice forward, causing a thinning as one moves upstream. This is particularly noticeable between Beardmore, Nimrod and Byrd Glaciers. We will consider this point later in more detail.

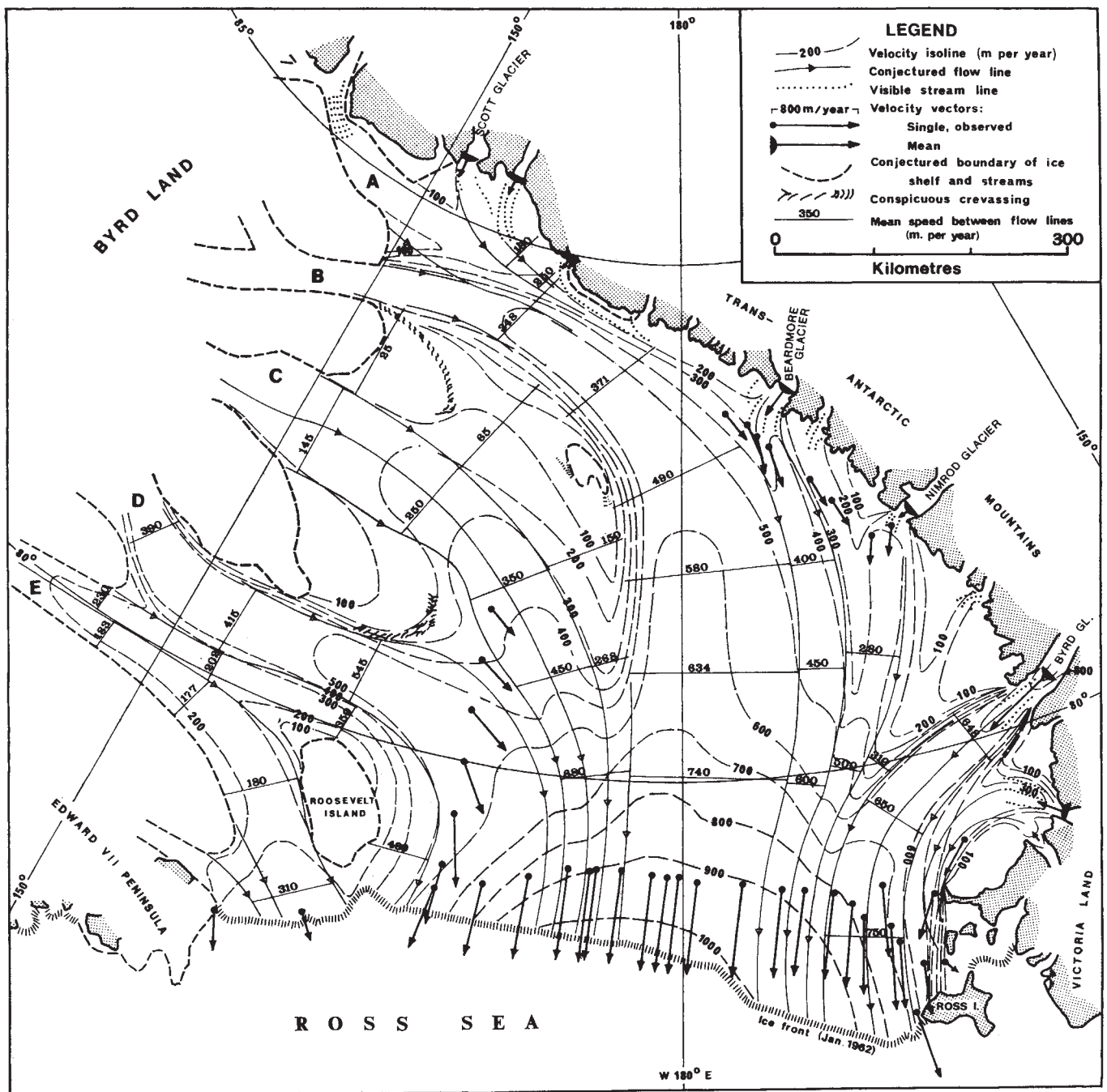


Fig. 2 Velocity field of ice flow.

Velocity distribution within the Ross Ice Shelf

Figure 2 shows the speed and direction of movement over the Ross Ice Shelf. Vector arrows show direct measurements of movement made by earlier investigators¹⁴⁻¹⁶. Streamlines of ice flow deduced from the thickness pattern and from calculations are shown by continuous lines, while dashed lines indicate lines of equal speed. Between 180° and 150°W streamlines were deduced entirely from the thickness pattern, and their direction agrees well with the direct observations of movement along 164°W.

We have assumed mass continuity of flow to extrapolate velocities upstream from Dorrer's velocities along 79°S (ref. 16). If $\bar{V}_1$ is the known mean velocity along a line 1 be-

tween two flowlines, the mean velocity $\bar{V}_2$ along line 2 between the same flowlines at a distance δx upstream is given by

$$\bar{V}_2 = \bar{V}_1 \frac{\bar{H}_1 W_1}{\bar{H}_2 W_2} - \bar{B} \frac{\delta x}{H_2} \left(\frac{W_2 + W_1}{2W_2} \right) \quad (1)$$

where $\bar{H}_1$ and $\bar{H}_2$ are the mean ice thicknesses and W_1 and W_2 the widths between the flowlines along the lines 1 and 2, and $\bar{B}$ is the mean mass balance per unit area over the area bounded by lines 1 and 2 and the flowlines. This mass balance must take account of accumulation and ablation on both upper and lower surfaces of the ice shelf. Observations by Cray and coworkers¹¹ show the distribution of net annual accumulation on the upper surface of the ice shelf, which

varies from 0.16 to 0.30 m yr⁻¹ of ice. We know that bottom melting up to at least 0.50 m yr⁻¹ occurs near the ice front while freezing may take place under the southern part. We assumed bottom melting of 0.12 m yr⁻¹ between 79°S and 80°S, falling off to zero between 83°S and 84°S, and we have made no allowance for any bottom freezing. The resultant mean velocities (m yr⁻¹) are shown in Fig. 2 above the lines to which they apply.

Although streamlines could readily be deduced from the thickness of the ice on the shelf between 150°W and 180°, between 180° and 160°E their location was less clear. In this section, flowline positions were adjusted through a process of iteration until calculated velocities near the Beardmore, Nimrod and Byrd Glaciers matched the observations.

The velocities between the pair of flowlines finishing either side of the 180° meridian are worthy of particular attention. Once the streamline flow in the south-eastern corner of the ice shelf had been determined by the 1971–72 flights with inertial navigation, it was clear that the major discharge of ice stream B passed to the south of the ice rise at 83°S, 172°W. As we knew the mass discharge of the Robert Scott Glacier and the Amundsen Glacier to the west, and could identify their flowlines to 165°W, we estimated the mean velocity of their stream at this point as 250 m yr⁻¹ compared with a mean value over the line also including the discharge of stream B of 348 m yr⁻¹. The velocity of the latter must therefore be around 500 m yr⁻¹. This result is particularly interesting for it explains why the rapid discharge of the stream B, which is only 800 m thick where it enters the ice shelf, dominates and rapidly bends the flow from the Robert Scott and neighbouring glaciers, although they are several hundred metres thicker on entering the ice shelf. In this area where there are strong thickness gradients, and hence greater surface slopes than elsewhere, we see that the slopes do not govern the movement. This is controlled mainly by the momentum of the stream with the largest mass discharge into the ice shelf.

Although a fully satisfactory three-dimensional solution to the problem of deformation within ice shelves has not yet been given, some relevant factors can be appreciated by considering the equations for quasi static creep of an ice shelf. We take the x axis to be oriented in the direction of flow and the z axis vertical. Then provided we are clear of local boundary effects and have no friction on upper or lower surfaces, we assume the shear stresses in the xz and yz planes to be zero, so the basic equilibrium equations⁶ reduce to

$$\frac{\partial \sigma_x}{\partial x} + \frac{\partial \tau_{xy}}{\partial y} = 0 \quad (2)$$

$$\frac{\partial \sigma_y}{\partial y} + \frac{\partial \tau_{xy}}{\partial x} = 0 \quad (3)$$

$$\frac{\partial \sigma_z}{\partial z} = \rho_i g \quad (4)$$

where ρ_i is the density of ice and g the gravitational force. The earliest theories of the flow of ice shelves^{1,2} were based on (4), while solutions including the effect of lateral drag either used the simplifying assumption⁵ that $\partial \tau_{xy}/\partial x = 0$, or⁶ that $\partial \tau_{xy}/\partial x$ was constant. We can see from equation (2) that as an ice stream is slowed down as it moves out into a uniform ice shelf, the change of stress in the direction of flow, and hence by simple theory of the ice thickness, must be balanced by a gradient of shear stress in the normal direction.

Similarly, any horizontal stress gradient normal to the flowline, which we expect to be present if the thickness varies across an ice stream, must be balanced by a gradient of shear stress in the flow direction.

We can summarise the position by saying that if an ice shelf is of uniform thickness, no differential stresses $\partial \sigma_x/\partial x$, $\partial \sigma_y/\partial y$, $\partial \tau_{xy}/\partial x$, $\partial \tau_{xy}/\partial y$ will be present. If the thickness varies in the flow direction, but is constant in a direction normal to flow, then $\partial \sigma_x/\partial x$ and $\partial \tau_{xy}/\partial x$ are not zero, and approximate solutions^{5,6} apply. But we must take account of all the four stress gradients if we are to obtain a solution to the problem of differential flow within an ice shelf.

Our results show that the largest thickness gradients occur normally to the flow direction on the sides of the major ice streams, and hence it seems that our maximum horizontal stress gradients $\partial \sigma_y/\partial y$ also occur here. From equation (3) we see also that the expected maximum values of $\partial \tau_{xy}/\partial x$ are in these regions. This fits the physical picture that the drag on the sides of an ice stream should vary most rapidly near its entry to the ice shelf. Although we cannot give a full solution, we see that as the shear gradually disappears as one moves further into the ice shelf, the thickness of the shelf becomes more uniform. Then, if we neglect other factors such as bottom melting and drag at the boundary of the ice shelf, we expect that as the ice stream approaches the same thickness as the ice shelf on either side, differential motion within the shelf will become small.

Our results indicate that the momentum with which an ice stream enters the ice shelf is perhaps more important than expected. The same process, but in an inverse sense, appears to explain the thicker ice to the south of Minna Bluff, the promontory that juts out into the ice shelf around 79°S to the south of Ross Island. But in this case it is the ice shelf streaming past the promontory that seems to provide the force that, rather than lateral drag, causes thickening in this area. The most complex situation is that caused by different ice streams entering the south-eastern corner of the ice shelf from different directions and at different speeds. Clearly a full solution for this case, both analytically and in terms of gathering adequate field data on all boundary conditions will be difficult. Similar considerations also apply near the ice front where the mechanics of calving of large tabular icebergs must depend on the same basic equations.

The value of the present work lies in the way in which the new data draw attention to these problems. Although predictions of the velocity of movement over the whole area of the ice shelf help our understanding of the region, the direct measurements of velocity that will be made in the near future by use of satellite position fixing methods will provide an interesting test of our main conclusions.

Work on radio echo sounding by the group at the Scott Polar Research Institute has been financed by the Natural Environment Research Council, and Antarctic flying operations have been organised by the US National Science Foundation. The work has been made possible by development of radio echo sounding methods by Dr S. Evans and others, and by support from the staff and aircrews of US Navy Air Development Squadron VXE6.

Received November 5, 1974.

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letters to nature

Outburst in U Cephei

A SYSTEMATIC survey of the profiles of the H α line in binary stars, shell stars, Be stars and related peculiar objects is being conducted by us (and G. J. Peters) with the Varo image intensifier attached to the coude spectrograph of the 120-inch telescope and the 24-inch coude auxiliary telescope of the Lick Observatory. Emission at H α is found to be a more common Phenomenon among the Algol-type eclipsing binaries than was thought before. Usually, but not always, the emission is best observed during the primary minimum when the light of the hotter and brighter component is either greatly reduced or completely occulted. This is in agreement with the models representing the Algol systems as evolved binaries in which mass is still being transferred from the late-type secondary component to the hotter primary star^{1,2}. Although the general picture is fairly clear, the problem remains how the on-flowing material and its angular momentum are distributed among the primary star, the disk revolving around it and a possible envelope enclosing the whole system. The purpose of our survey is to furnish quantitative data on this process.

Observations of U Cephei show that the mass transfer and disk formation may be a very variable process. Batten³ and coworkers have tried to detect emission in this system, but found either no emission or at best a marginal phenomenon. On August 8, 1974, however, we observed very strong H α emission during a primary eclipse. Photometric observations by L. McDonald accurately determined the time of mid-eclipse (1974 August 8.4345 = JD 2442267.4345 heliocentric) and the phases.

Our equipment gives a very good dispersion (17 Å mm⁻¹) and good time resolution (exposure times were 5 to 20 min), so that the behaviour of the emission line can be studied in detail. Figure 1 shows the density tracings of nine consecutive spectrograms and Table 1 gives the peak intensities of the violet and

red emission lobes as functions of phase. Our timing of the exposures was very fortunate: the first plate coincided with the beginning of the total eclipse, the third plate with mid-eclipse, and the fifth plate with the end of the total phase. Five more spectrograms were obtained in the ensuing partial eclipse. All the plates show a very strong, deep and relatively narrow central absorption core at H α . During and near the total phase, this core, and several weaker absorption lines, were the result of the secondary (G8 III-IV) component. Emission lobes appear on both sides of the H α absorption core. Contrary to the Victoria observations made in September, however, no other emission lines were observed, although some suitable lines of Fe I, Fe II, and He I do lie in the observed spectral region around H α .

Table 1 Peak intensities of emission lobes

Plate	Phase d	Fraction of period (P)	Peak intensity (continuum = 1.00)	
			Violet lobe	Red lobe
EC 12579	-0.0302	0.9879	1.07	1.49
12580	-0.0135	0.9946	1.13	1.33
12581	0.0012	0.0005	1.20	1.22
12582	0.0200	0.0080	1.33	1.15
12583	0.0337	0.0135	1.35	1.09
12584	0.0464	0.0186	1.30	1.07
12585	0.0573	0.0230	1.28	1.06
12586	0.0686	0.0275	1.20	1.05
12587	0.0803	0.0322	1.10	1.01
12588	0.0873	0.0350	1.02	1.00

At the beginning of the total phase, the red emission lobe is very strong, while the violet lobe is marginal but real. As the eclipse advances, the red lobe weakens, while the violet lobe grows in strength. Both are equally strong at mid-eclipse, and the H α profile is symmetrical. The violet lobe reached its greatest intensity near third contact, but never quite matched the peak intensity of the red lobe on the first plate. This suggests greater accumulation of material on the impact side of the primary star. As the primary component gradually emerges from the total eclipse after phase 0.02P, both emission lobes decline in intensity, but the violet lobe remains stronger.

Qualitatively, this sequence of events agrees with the model of a rotating ring around the primary component, originally proposed by Joy⁴ for RW Tauri. For the first time, however, the dispersion and time resolution have been so good as to permit a quantitative analysis of the profiles, which is under way.

This complete ring—or better disk—must have been formed very recently. Previous observations⁵ indicate only a 'bridge' of material between the two components⁶, while Huang and Struve⁶ predicted that emissions in U Cephei and a few other similar systems should occur intermittently. It is probable that the primary cause was an outburst of the secondary component, with the ensuing increase in the rate of mass transfer. If so, photometric timings of future minima should show a jump in the period.

We suspect a real change in the structure of the disk within the month that elapsed between our observations and those by Batten *et al.*⁷. The seven-colour intermediate-band photometric observations made by L. McDonald simultaneously with our spectrographic observations show distortions in the light curve near the onset and the end of the total eclipse. The duration of

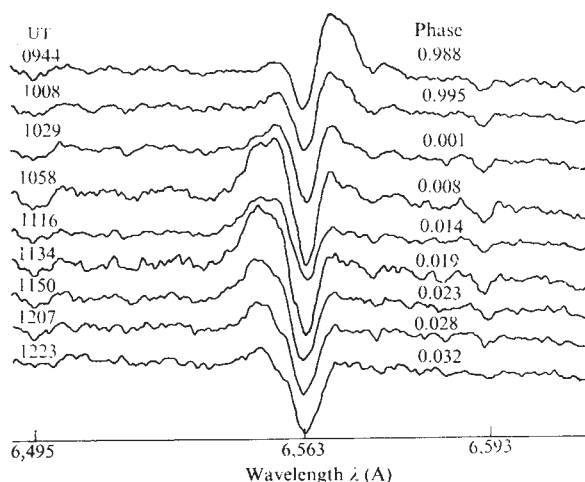


Fig. 1 Density tracings of the H α region on nine spectrograms taken on August 8, 1974. Tracings are superimposed so that the absorption cores nearly coincide. Laboratory wavelengths in Angstroms are indicated on the x axis.

the total phase seemed to be much shorter than usual, but in most colours this cannot be simply interpreted as the consequence of an increased photospheric radius of the primary star. Rather, one must invoke a 'third light' in the system. Somewhat similar but weaker bumps in the light curve of RW Tauri observed by Grant⁸ were interpreted by him as the light of the emission lines. Line emission cannot be responsible for the entire effect in U Cephei.

Outbursts of this type may be fairly frequent in the Algol-type binaries, and their observations should contribute greatly to our understanding of the mass transfer processes in close binary systems.

We thank L. McDonald for preliminary information on his photometric observations, H. L. Burger for assistance, and R. C. Crawford for the Figure.

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Received November 25, 1974.

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Outburst of U Cephei

WE believe that our recent observations of the eclipsing binary U Cephei have implications for the study of the evolution of binary systems and the understanding of novae.

The system U Cephei contains a B7 V star of mass $4.2 M_{\odot}$ and radius $2.9 R_{\odot}$ and a G8 III-IV star of mass $2.8 M_{\odot}$ and radius $4.7 R_{\odot}$. The radius of the circular orbit is $14.7 R_{\odot}$, and the orbital inclination is close to 90° (ref. 1). The orbital period of approximately 2.5 has been increasing throughout the nearly 100 yr that the star has been recognised as an eclipsing binary. The average rate of increase is about 5 parts per 10^6 per cycle, but there are irregular abrupt period changes superimposed on the average increase, and the present rate of increase is faster. The average rate can be explained as a result of transfer of matter from the G8 star to the B7 star, and is one of many pieces of evidence for the existence of gas streams in this system.

During the total eclipse of the primary star, in systems of this sort, one can often observe emission lines superimposed on the spectrum of the secondary star. These lines originate in the gas streams, but in spite of the strong evidence for such streams in U Cephei, emission of this kind has been reported in the spectrum only once², and that was very weak. During the eclipse of September 7, 1974, however, very strong emission was observed by one of us (B.W.B.) in the course of a routine programme of observation of this and similar systems. The emission was clearly present in all Balmer lines from H β to H18 (Figs 1 and 2b), and also in the H and K lines of Ca II, $\lambda 4481$ of Mg II, and possibly in some lines of Fe II and He I. The changing intensities of the red and violet components during the course of the eclipse suggest that the emission arises in a disk that rotates around the primary star with an average velocity close to 250 km s^{-1} (Fig. 1). This emission persisted and was seen during all

the observable eclipses in September and early October. By October 17, however, it had appreciably weakened. During the same time, emission was visible at H α even in full light. For about a week in the middle of September this H α emission was accompanied by a sharp absorption component, which could also be seen at H β , H γ , and H δ , with a displacement to the violet corresponding to a velocity of several hundred km s^{-1} (Fig. 2a). This component shows large variations; on September 15, about 0.25 P after primary minimum, its displacement corresponded to -620 km s^{-1} , on September 16 at 0.67 P to -350 km s^{-1} , on September 21 (at almost the same orbital phase) to -250 km s^{-1} , and on September 22 (at the end of primary eclipse) to approximately -700 km s^{-1} . The disk around the primary star seems to have been expanding, and some matter probably left the system. During September the spectrum of the primary star changed; lines of He I almost disappeared, while those of Ca II, Mg II, and Si II became stronger. Lines of Fe II previously

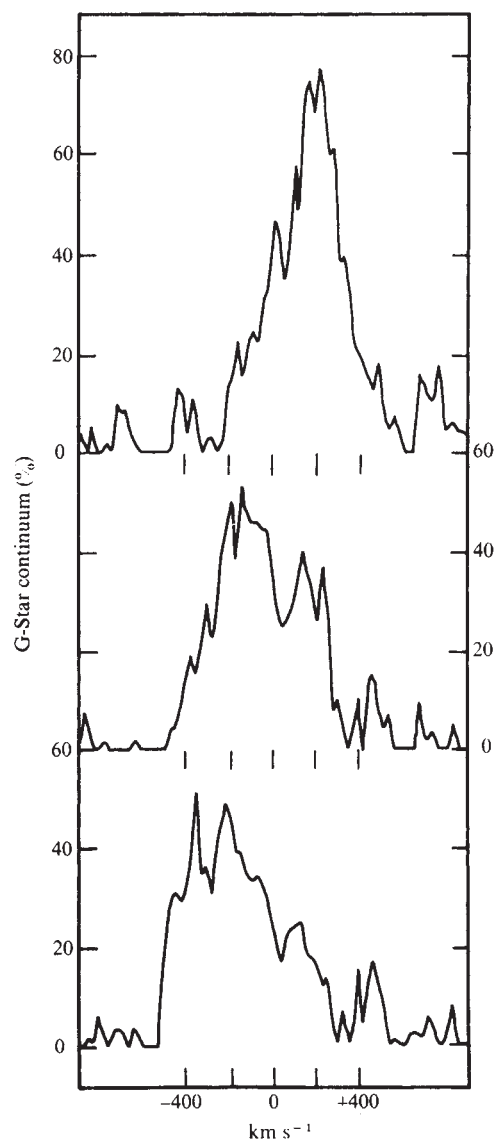


Fig. 1 The change of emission intensity at H β with phase during the eclipse of September 7, 1974. The normal emission-free G-type spectrum has been subtracted from the observed spectra. Exposure times for each plate were about 45 min. The upper (first trace) was centred about 1 h before, the second (middle) about 10 min before, and the third about 40 min after the estimated time of mid-eclipse. The horizontal scale, referred to the rest position of H β is approximate.

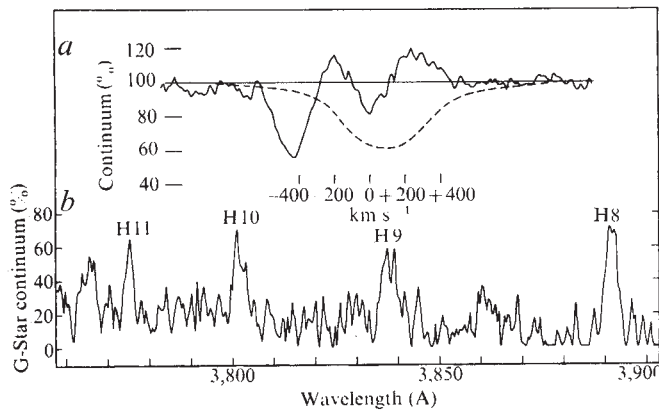


Fig. 2 *a*, Rectified intensity tracing of the line profile of H α on September 16, 1974, referred to the continuous spectrum in full light. The zero of the velocity scale is the approximate rest position of H α . The dashed symmetrical profile is the normal absorption profile of H α displayed by an amount appropriate to the orbital phase. The displacement of the violet absorption, over 400 km s⁻¹, is greater than the amount quoted in the text which is the mean for the lines H α , H β , H γ , and H δ . *b*, Rectified intensity tracing of a region of the spectrum of U Cephei made from a plate obtained about one hour before mid-eclipse on September 7, 1974. The normal emission-free G-type spectrum has been subtracted from the observed one, but there is still some contribution from the B-type spectrum. The wavelength scale is approximate.

invisible appeared in the spectrum; especially prominent amongst them is the triplet $\lambda\lambda 4924, 5018, 5169$ which is normally barely visible but which was also seen in October 1969 when emission was last observed in the system. The spectral type is more like B9 or A0 (the original Henry Draper type) than the normal B7. Photometric measurements out of eclipse in late September showed that the $(B-V)$ colour of the primary star had changed to 0.0 mag from its customary value of nearly -0.1 mag. The $(U-B)$ colour, however, had not greatly changed.

Photometric observations during eclipse show a changed form of light curve that has also been observed by Plavec and Polidan³ and by Olson⁴ and which is illustrated in Fig. 3. We believe that the abrupt change of slope during ingress is real, but we cannot yet explain it. Note that totality is shorter than normal, but the duration of the whole eclipse seems longer than usual. During totality, the V magnitude and $(B-V)$ colour of the secondary star are normal (as is also the secondary spectrum). The $(U-B)$ colour is different; the secondary component has always shown an ultraviolet excess (compared with other G8 III-IV stars) of about 0.1 mag. During this outburst the excess has been up to 0.2 mag greater, that is $(U-B) = 0.25$ mag. We believe that this is a decisive argument against ascribing the excess to metal deficiency of the secondary star. There is some indication that the system as a whole is slightly fainter than usual out of eclipse, but the difference does not exceed 0.05 mag, and because our photometric system is not precisely the U, B, V system we are not sure that the difference is real.

The key to our interpretation is that although totality is shorter than usual, during the total phase nothing except the U magnitude is different. The secondary star, therefore, is in its normal state. If the primary star has formed a new effective photosphere, appreciably larger than the normal one, then both the decrease in the duration of totality and an increase in that of the whole eclipse would be inevitable. The new photosphere would have to have a lower temperature than the old one did, because the out-of-eclipse brightness of the system has not greatly changed. The duration of totality differs from eclipse to eclipse, and even from colour to colour at the same eclipse. On September 12, totality lasted about 50 min (0.014 P) instead of just over 2 h (0.037 P)—the normal value. Since the sine of the

phase angle of second contact is given by the difference in the radii of the two stars divided by their separation, and the radius of the secondary star is apparently unchanged, these figures imply a radius for the effective photosphere of 4.1 $R_{\odot}$ —an increase of 42%. If the radiating surface is to increase by this amount without changing the total luminosity of the system, its temperature must be decreased by a factor 0.84. According to Morton and Adams⁵, a B7 V star has an effective temperature of 13,600 K, and this must therefore be reduced to 11,400 K—appropriate to a spectral type between B8 and B9 and not inconsistent with the changes observed in the spectrum and the $(B-V)$ colour. The proposed effective photosphere could have been created either by an exceptional ejection of matter from the G8 star, or by an expansion of the outer layers of the B7 star. We incline to the latter possibility because of the evidence for the expanding shell, and the apparent normality of the G8 star. The instability of the B7 star may, however, have been triggered by an earlier unobserved eruption of the G8 star.

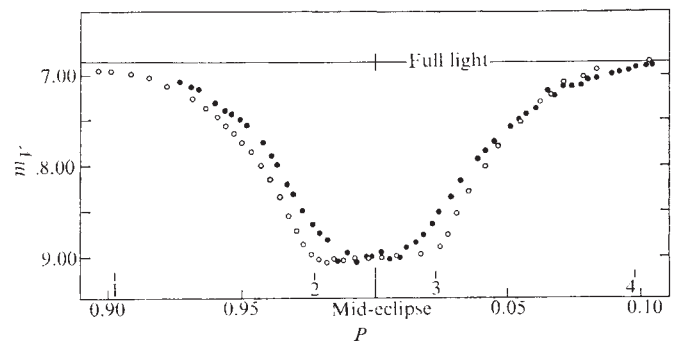


Fig. 3 Photometric observations of U Cephei with a V filter on September 27, 1974 (filled circles) compared with normal points derived from similar unpublished observations by Broglia in 1957-8 (open circles). Observations from Victoria in 1971 and 1973 fit Broglia's light curve perfectly, but do not cover it completely. Times of the four contacts for Broglia's curve are indicated on the time axis. The times of mid-eclipse, determined separately for each light curve, have been made to coincide. The line indicating full light at 6.85 mag refers to Broglia's curve. Note that even the undisturbed light curve is asymmetric.

Our observations and those of ref. 3 show that U Cephei has been active throughout August, September, and part of October 1974. Spectral scans made in early July by Rhombs (private communication) suggest that some changes were taking place even then. Photometric and spectroscopic observations by us in September, 1973, showed the system to be normal and without emission then. The photometry was confirmed at the end of September, 1973, by Coyne⁶. So the outburst could not have begun earlier than October, 1973, nor later than July, 1974. Our most recent observations suggest that it is ending in October, 1974: the emission is weaker, the lines of ionised metals are disappearing from the primary spectrum, and the light curve has more nearly its usual shape. We believe we may have observed the mechanism of an abrupt period change in progress. Bakos and Tremko⁷ have reported a much smaller distortion of the light curve that they observed in August, 1969, a few weeks before emission was first detected in the spectrum of U Cephei. Hall⁸ has also reported large changes in the light curve of RW Persei. The detailed spectroscopic and photometric coverage of this incident by several independent groups is so far, however, unique in the study of close binary systems.

Further study of our observations will undoubtedly improve our understanding of the process of mass transfer in systems of this kind. We also believe it will have implications for our understanding of novae. The expanding shell and the larger and cooler

temporary photosphere are found in novae. The binary nature of many ex-novae has been demonstrated, and it is generally accepted that nova eruptions are in some way linked to the presence of a close companion. Most theories are based on the assumption that the seat of the eruption is a degenerate star (see ref. 9). The present observations suggest that something similar can take place in a system containing a main-sequence star.

We thank the observers who helped to obtain spectrograms.

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Received November 25, 1974.

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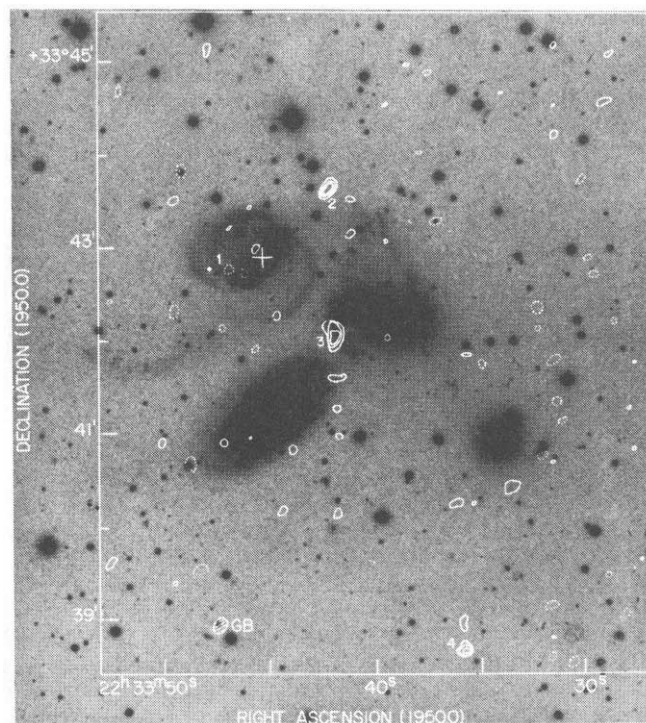


Fig. 1 The 2.695 GHz observations are superimposed on a 200-inch print of Stephan's Quintet. The cross represents source No. 1 which has been subtracted from the map. The synthesised beam is given in the lower left corner. The outer 2.695 GHz contour level is at 1.2 mJy (1 r.m.s. = 0.5 mJy) increasing thereafter in intervals of 0.8 mJy.

High frequency radio observations of the Stephan's Quintet region

RADIO observations have been reported for several apparently interacting astronomical systems^{1,4}. These observations do not provide conclusive data as to the frequency of occurrence of radio emission from multiple systems compared with that for normal galaxies. Although the detected radio fluxes do not generally fall in the 'radio galaxy' range, there is evidence that for some interacting systems at least, there is a statistical excess of the number of radio sources in their vicinity^{5,6}. The aperture synthesis observations mostly show sources coincident with member galaxies; a few show emission from regions between the galaxies. In the case of Stephan's Quintet, observations⁷ with the Westerbork Synthesis Radio Telescope (WSRT) at 1.415 GHz suggest an arc-shaped source partially encircling a point source centred on NGC7319.

The National Radio Astronomy Observatory three-element interferometer was used at frequencies of 2.695 and 8.085 GHz, between January 1 and July 3, 1973, to synthesise areas of $18' \times 18'$ and $6' \times 6'$ respectively, centred at $\alpha = 22^{\text{h}} 33^{\text{m}} 48.0^{\text{s}}$, $\delta = 33^{\circ} 41' 00''$ (1950) near Stephan's Quintet. Observations were obtained at 16 telescope spacings from 100 to 2,700 m. The resulting synthesised beamwidths at the two frequencies are $9''$ by $6''$ and $3''$ by $2''$ respectively, (both at position angle 324°), with the close-in side lobe levels less than 30%.

In the present observations, the point source of ref. 7 is detected at both frequencies but appears somewhat extended. This source was successfully subtracted from the synthesised map by a model at 2.695 GHz composed of a 10.4 mJy point source, with a 3.1 mJy slightly extended component $1''$ east

and $3''$ south of it. The same model with fluxes of 2.5 and 1.3 mJy respectively, fits the 8.085 GHz observations; the latter flux is more than four times the root mean square flux at that frequency. In the subtraction procedure used, no single point source in the vicinity sufficed to remove the observed source; the two component model was the best at minimising the residuals. This indicates that the source is definitely extended. Furthermore, the crescent-shaped source is clearly resolved at the lower frequency into two extended sources, roughly coincident with the peaks seen in the map of ref. 7. Both sources are below the detection limit at the higher frequency.

In Fig. 1, we present the results of our 2.695 GHz observations, superposed on a 200-inch photograph provided by H. C. Arp. This superposition is based on an overlay program for the Palomar Sky Survey, which, because of the small scale of the latter, can result in a displacement error as large as $15''$ in spite of the much higher positional accuracy of the interferometric observations. As in the WSRT map, our source No. 1 is centred on NGC7319. Source No. 2, corresponding to the northern source of the crescent of ref. 7, seems to lie on a blank field. But source No. 3, the southern source, falls quite near the HII emission features seen by Arp⁸ in the spiral arm of NGC7318b. Source No. 4 seems to be quite near a small faint compact galaxy. The slight discrepancy between our positions for sources 2 and 3 and those shown on the overlay of ref. 7, can be explained by the displacement error.

Table 1 Source data

Source	RA (1950.0)	Dec. (1950.0)	$S_{2.695}$ (mJy)	$S_{8.085}$ (mJy)	$\alpha_{2.695}^{1.400}$ (± 0.3)	Remarks
	(± 0.19 s)	($\pm 2''$)	(± 0.5)	(± 0.3)		
1	22 h 33 min 45.9 s	+33° 42' 57.8"	13.5	3.8	1.1	Source on nucleus of NGC7319
2	22 h 33 min 42.2 s	+33° 43' 39.9"	3.2	< 1.5	< 1.7	Source within the crescent
3	22 h 33 min 42.2 s	+33° 42' 02.9"	5.1	< 1.5	< 1.7	Source within the crescent
4	22 h 33 min 35.4 s	+33° 38' 54.0"	5.5	—	1.5	Out of 8.085 GHz field of view

Our observations do not reveal the arc structure seen in the WSRT map. In order to examine the possibility that the arc may have resulted from a blending of our two sources in the lower resolution map, we have convolved our observed map with a larger beam approximating the WSRT resolution. The resulting map still showed the two sources well resolved. We conclude that the apparent discrepancy results from either of two possibilities. Firstly, that such an arc does indeed have a continuous distribution as seen in the WSRT map, but that the continuity is broken at our higher frequency by the weakness of the emission at all areas other than the peaks. If the whole arc structure has as steep a spectrum as the two sources, (as seen in Fig. 2), then the flux density level received from the rest of the arc would never exceed three times our r.m.s. level. Secondly, the structure may be a blend of four or more sources, of which only two are within our detection limit at 2.695 GHz.

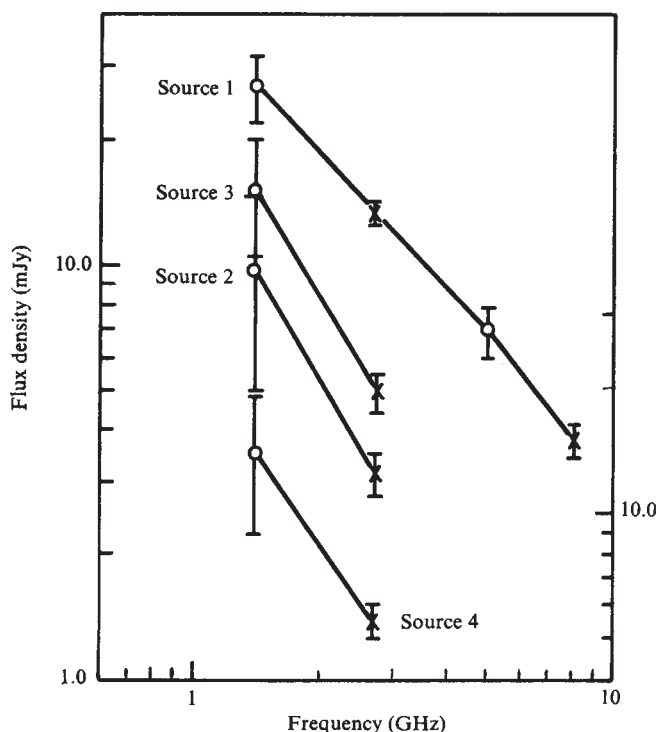


Fig. 2 Spectra of sources in Stephan's Quintet area. ×, present observations; ○, WSRT observations⁷.

No distinction between these two alternatives can be made with our present observations.

Table 1 shows the positions, fluxes, and spectral indices, α , for sources detected within the larger area observed. The r.m.s. errors are listed at the top of the respective columns. All fluxes have been corrected for gain of the primary beam.

In Fig. 2 we show the spectra of the three resolved sources seen within the quintet and the fourth source detected about 5' south preceding NGC7319. The 4.995 GHz flux value for source 1 is from preliminary observations by Allen (unpublished). In the case of source 4, the 1.415 GHz peak flux was read from a full field map provided by Allen (unpublished).

The most striking feature of the spectra is their steepness. Indeed, with the exception of source 1 (which falls on NGC7319), all three indices exceed the 1.3 limit observed for most extragalactic sources⁹. It is tempting to speculate that the extreme steepness of these spectra together with the extended forms of the sources may indicate that they are remnants of an earlier ejection, at least for the sources in the arc, and that the source centred on NGC7319 is of a later epoch. It should be pointed out, however, that the extreme steepness of sources 2 and 3

would be somewhat reduced if some flux were present due to an extended component that we failed to detect.

An interesting and less speculative conclusion results from the comparison of single dish observations of the area with the present high resolution measurements. Two of us⁶ have noted the probable existence of a ridge of radiation in the area of Stephan's Quintet at low frequencies. Since the present interferometric observations are insensitive to extended sources, certainly beyond about 3 arc min in extent, any excess found in a single dish measurement over the sum of the small sources falling within its beam would confirm the existence of such a ridge. Such single dish measurements within the spectral range shown in Fig. 2 (eliminating the need for extrapolation) have been made by Arp¹⁰, at a frequency of 2.295 GHz. Interpolation of the fluxes for the four sources, all falling within the beam of Arp's measurements gives a value of 34 ± 2 mJy. A similar interpolation using the whole extended arc of WSRT gives a value of 40 mJy. Arp's single dish measurement gives a value of 90 ± 10 mJy, well over twice the value expected from either of the above interpolations. We thus conclude that the evidence for a structure of ≥ 3 arc min in extent suspected at low frequencies is strengthened by the present results which strongly support the existence of a broad component at the higher frequencies.

We thank Dr R. J. Allen and Professor H. Arp for their comments and the former for providing us with preliminary data before publication. One of us (M.A.K.-K.) was partially supported by an NSF Travel Grant. NRAO is operated by Associated Universities, Inc., under contract with NSF.

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Received September 23; revised November 21, 1974.

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Seismographic observation at the bottom of the Central Basin Fault of the Philippine Sea

THE Central Basin Fault of the Philippine Sea is proposed as an extinct mid-oceanic ridge^{1,2} and seems to be a key to the development of the floor of the Philippine Sea. Though the fault seems to be aseismic on the basis of land networks, it is interesting to know whether microearthquakes occur in the vicinity of the fault. We put a sensitive ocean-bottom seismo-

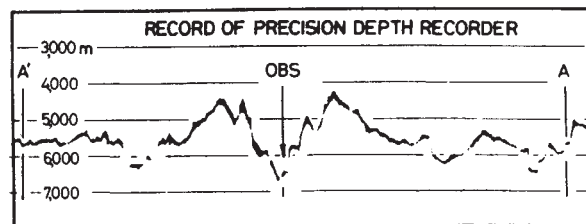


Fig. 1 Locations of observation sites of ocean-bottom seismographs. Bathymetric map (depths in fathoms) is reproduced from HO 1301 publication. Intersection lower left is a record of ship-borne depth recorder of RV Hakuho-maru. Position of A: $15^{\circ}06'N$ ($15.10^{\circ}N$), $129^{\circ}54'E$ (129.91°). Position of A': $16^{\circ}43'N$ ($16.71^{\circ}N$), $131^{\circ}15'E$ (131.24°). Direction from A to A': 39° (perpendicular to the fault); distance from A to A': 233 km.

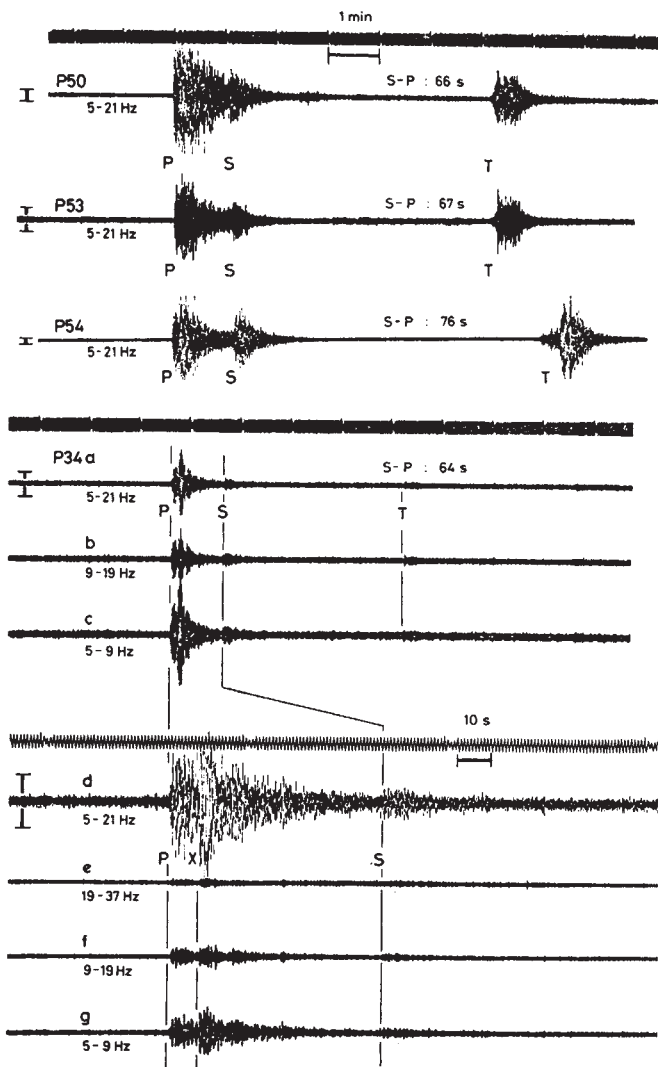


Fig. 2 Some earthquakes recorded by OBS. Number of events, frequency band of band-pass filters which were used in reproduction off magnetic tapes, S-P time as indicated. T phase travel time data support the view that the velocity of T is 1.5 km s^{-1} . Equivalent ground velocity amplitudes of $15 \times 10^{-6} \text{ cm s}^{-1}$ are also shown by bars on each trace. The X-phase is discussed at the end of the paper.

graph (OBS) in the median valley of the ridge (the width of which is only a few kilometres) and from its recordings have been able to deduce the existence of microearthquakes. It has also been possible to estimate P and S velocities for the top of the mantle and a Q structure for the upper mantle.

Seismographs have been developed over the past few years^{3,4} for seismographic survey in the vicinity of deep sea trenches of the western Pacific⁵ and for long-range explosion experiments in ocean basins⁶. The instruments were intended to record low level seismic signals and were designed to be buried in the ocean sediment to avoid the noise generated by ocean-bottom water currents.

The position of the bottom seismography (by satellite navigation) was 16.03°N , 130.72°E (Fig. 1). The depth was 6,500 m and the bottom was covered by sediment. The recording period was about 63 h, from 0930 GMT on September 16 to 0030 GMT on September 19, 1973. More than 120 earthquakes were recorded, none in the vicinity of the observation site. Considering the sensitivity of the seismograph, no earthquake whose magnitude was more than -3, 0 or 1 occurred within 20, 100 or 250 km of the observation site. All the earthquakes observed, except one, took place beyond the edges of the Philippine Sea

plate as they have S-P times of more than 64 s. The exception was an earthquake with an S-P time of 25 s, and a magnitude of about 2.

The numbers of earthquakes recorded, 2 h^{-1} , and the distribution of S-P times indicate high sensitivity of the bottom seismograph (the distances from epicentres being more than 700 km) and a high level of seismicity along the Philippine and Ryukyu trenches.

Travel times of an earthquake near Taiwan, located by the WWSSN network (0709 GMT on September 18, 24.9°N , 122.0°E , $h = 86 \text{ km}$, $M = 4.9$), show that the mean V_p value is 7.9 km s^{-1} and mean V_s value is 4.6 km s^{-1} , when the epicentral distance is 1,340 km. The mean V_p value is considerably lower than the 8.2 or 8.3 obtained in the western Pacific Basin for similar epicentral distances (T.A., H.S., Y.T., and K. Kobayashi, unpublished). The S-P velocity is 11 km s^{-1} . The value of V_p indicates that the upper mantle velocity beneath the Philippine Sea is considerably smaller than that of normal ocean basins.

The seismograms we recorded have strangely small amplitudes of S phrases (Fig. 2). Although only a vertical seismometer (natural frequency 3 Hz) was used in this observation, the marked diminution of amplitudes of S waves contrasts markedly with seismograms of similar S-P times recorded at the other observation sites of our bottom seismographs. It is probable that shear waves are greatly absorbed in the upper mantle beneath the Philippine Sea; this perhaps results from partial melting not unconnected with high heat flow data (Y. Kono, and Y. Tomoda, personal communication).

Spectral analyses of the initial part of P waves were performed. If we assume the source spectra of earthquakes, the mean attenuation factor $\bar{Q}$ along the wave path can be calculated from the slopes of spectral curves (Fig. 3). Differences in the assumed source spectra probably result in a trifling difference in the estimation of $\bar{Q}$ because magnitudes of earthquakes do not differ by much in this study. The estimated $\bar{Q}$ values are shown

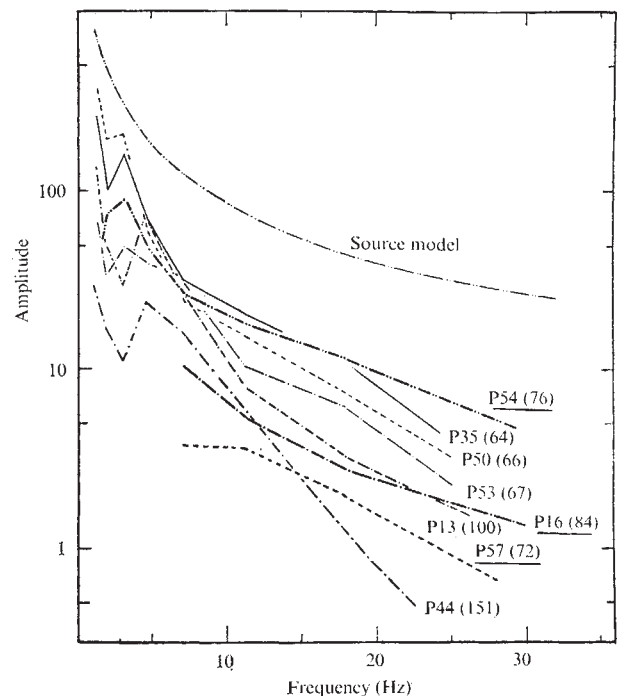


Fig. 3 Some examples of frequency spectra of first part (initial 3 s) of P waves. Number of event and S-P times (in parentheses) are shown at each curve. Note that high frequency components of earthquakes with S-P times of 70-80 s are more abundant than those of 64-67 s. One of Aki's source models⁵ is also shown.

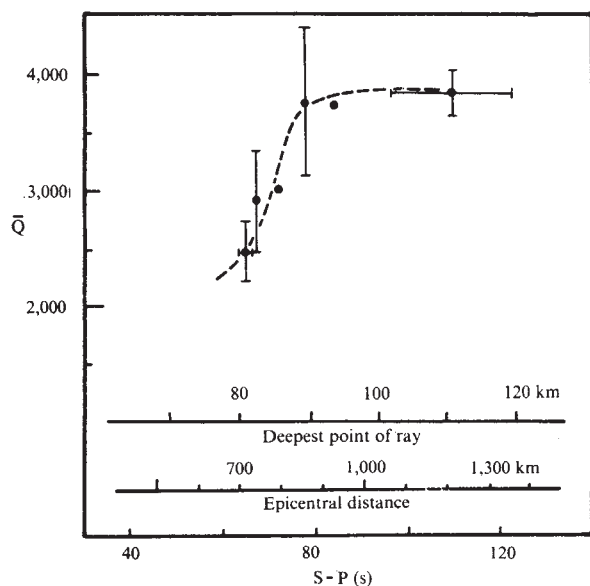


Fig. 4 Distribution of $\bar{Q}$ values. In the computation, the ω^{-1} model for the source of these frequencies (or model B of Aki⁵) was used. Although other source models will produce different absolute values of $\bar{Q}$, an abrupt increase of $\bar{Q}$ value at the same range and the same depth is observed.

in Fig. 4, illustrating an abrupt increase in $\bar{Q}$ value at a distance of about 800 km. This suggests that a high $\bar{Q}$ layer exists. The depth to the boundary of this steep increase of $\bar{Q}$ value cannot be accurately estimated because the velocity structure is not known; nevertheless it is about 80 km if we assume the Japanese Meteorological Agency travel timetable, which fits well in and around Japan. Although $\bar{Q}$ values of about 2,000 are higher than those of island arcs such as Honshu, the values are still considerably lower than those of ocean basins obtained from other OBS observations.

Most of the recorded seismograms have clear secondary arrivals at 8–9 s after onsets of P waves (see the X-phase on Fig. 2).

This peculiar phenomenon can be explained if the secondary waves are P to S conversions at the boundary at which $\bar{Q}$ value is abruptly increasing. The highly attenuative zone underneath the western part of the Philippine Sea is probably bottomed by a high $\bar{Q}$ layer at a depth of about 80 km. This structure is quite different from that of the north-west Pacific Basin (T. A., H. S., Y. T., K. Kobayashi, S. Murauchi, N. Den, and T. Asanuma, unpublished) and could be peculiar to extinct mid-oceanic ridges.

We thank the officers and crews of RV Hakuho-maru of the University of Tokyo for help in laying and recovering the OBS.

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Bounds on the P velocity for the whole Moon

THE hedgehog algorithm suggests that either some Apollo seismic travel time data are in error (some layers dip) or a high velocity lid exists at a depth of 60 km in the Moon. The velocity depth curves for the Moon derived from the Apollo seismic data^{1,2} are probably not representative of the Moon as a whole and lateral inhomogeneity in the near surface layers can affect the generality of measurements of arrival time and amplitude.

We are particularly cautious about amplitude data, as plausible lateral variation in the near surface layers of the Moon can result in substantial errors in the estimates of ray

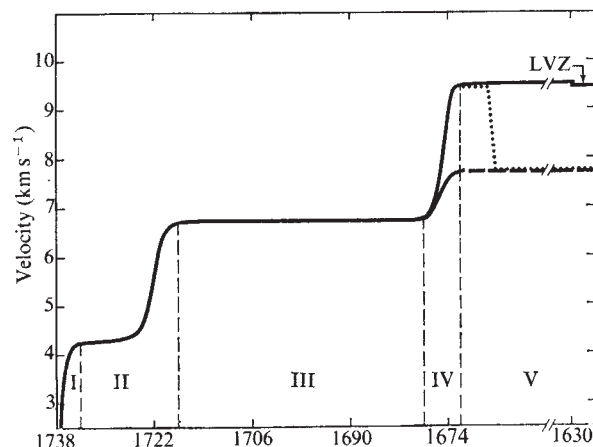


Fig. 1 Initial models for a hedgehog search of lunar structures. —, Class A; — —, class B;, class C.

parameter based on a radially homogenous Moon. Since amplitudes depend on derivatives of ray parameters we suggest that the use of amplitude data to elucidate lunar structure is premature.

The first few kilometres of the Taurus-Litrow region of the Moon have a step-wise velocity depth structure compatible with a series of lava flows³. Travel time differences of up to 1 s can arise if the thickness of any lava layer

Table 1 P wave travel times used in the inversion

Delta (degrees)	Travel time (s)	Weights
0.287	5.57	4.0
2.2	17.8	1.75
3.07	22.0	1.75
3.76	25.0	2.0
4.45	28.6	2.0
5.18	32.0	2.0
5.67	35.7	2.0
6.07	26.6	2.0
11.2	57.0	2.0
11.8	55.0	2.0
—	61.0	2.0
28.0	123.1	2.0
34.0	155.1*	2.0
	151.0*	1.5

*Only one of these two arrivals was used; in class B models, which one is used affects the half space velocity acceptance region by about 0.5 km s⁻¹.

is doubled or halved. As such variations would introduce errors in any analysis which considers the Moon to be laterally homogeneous, we add them to those observational errors which we have estimated as 0.5 s from a few sample seismograms¹. We considered variable accuracy by assigning weights based on discussions in the literature^{1,2}.

Solution of the inverse problem for such a set of travel time observations is well suited to the hedgehog algorithm⁴. This algorithm can deal with amplitude data and with travel times. Until the effect of intensive scattering is understood and synthetic seismograms can be made to fit substantial portions of lunar seismograms, however, we hesitate to use such data.

We used three models (Fig. 1) as a starting point in our search for a range of models which would fit the data within the accuracy of the experiment. The criteria for fit are: (1) the root mean square error of fit shall be less than σ ; (2) no more than j time observations shall differ from computed times by k times the weight W_i of the i th phase.

Table 2 P wave travel times*

Delta (degrees)	Travel time (s)	Weights
1.1	10.5	2.0
1.5	14.0	2.0
1.9	17.5	2.0
5.18	35.5	2.0
11.2	54.0	2.0

*Note the considerable uncertainty in these points. They were read from a graph of travel times prepared by Toksoz *et al.*¹.

We used various values for σ , j and k , and coarse searches in which positions were changed by increments of 10 km and velocities by increments of 1 km s⁻¹, together with fine searches with increments of half this size. We could not find any acceptable models of class A (Fig. 1) with any reasonable set of σ , j , k values. The data for the triplication (from either Table 1 or 2) suggest a half space velocity of 9.5 km s⁻¹ while the travel times near 30° suggest a velocity of 7.75 km s⁻¹.

Class B models all have a poorly resolvable low velocity zone (LVZ) at a depth of about 100 km. With an LVZ

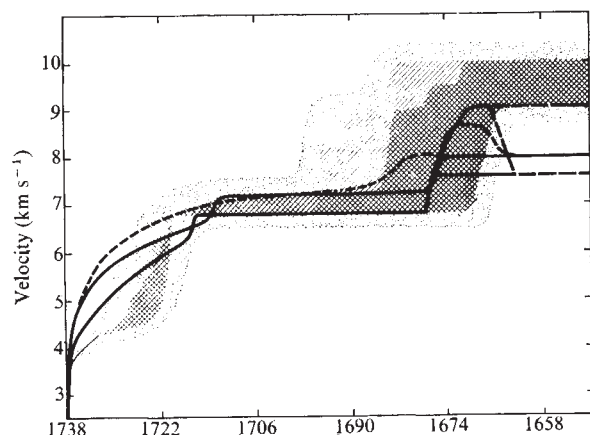


Fig. 2 Various solutions for the lunar velocity depth curve. The lines are published^{1,2} solutions using both amplitude and travel time data. The cross-hatched area is the region delineated by a fine hedgehog search. The lined area plus the cross-hatched area is derived from the coarse search. The stippled area illustrates the uncertainty caused by discrete steps taken in the hedgehog search.

Table 3 P wave travel time data at large deltas

	Delta (km)*	Travel time (s)	Weight
Meteoroid impact of day 134, 1972	967	131.5	1.0
	1,026	138.5	1.0
Apollo 16 SIVB	1,099	147.0	0.4
From Table 1	850	123.0	2.0
	1,032	151.0	2.0
		155.1	2.0

*30.3 km = 1° on the lunar surface.

These observations depend in part on an assumed model of the Moon's mantle.

located at this depth we have no direct P arrivals near 30°. Multiples (for example, PP) may be possible. If the arrivals at about 1,000 km are PP: (1) Using data in Table 1, together with the acceptance criteria ($\sigma=2.8$, $j=0$, $k=1.2$) and a coarse grained search, we found some acceptable structures (Fig. 2). (2) Using data in Tables 1 and 2 ($\sigma=6.0$, $j=0$, $k=2.4$) and a fine search we found additional acceptable structures. (3) Using data in Tables 1, 2 and 3, no acceptable solutions were found in a search based on ray theory alone. If the uncertainties (σ and k) are reduced to half the values in cases (1) and (2) there are no acceptable solutions on the basis of a fine search. If the data in Table 3 are in error by more than 10 s, solutions (1) or (2) are correct; otherwise, models A and B are inadequate. If class B models and Fig. 2 are accurate, high velocities in the first half space raise strict petrological constraints¹. Class C models are characterised by a thin lid of high velocity. Classical ray theory is not applicable in a thin layer. We expect, instead, a head wave in the lid whose low frequency components will tunnel into the low velocity zone beneath it. This tunnelled phase will be effectively low-pass filtered while the headwave is high-pass filtered.

The anomalous data at 1,000 km can be interpreted as a combination of tunnelled waves and head waves⁵. The velocity at the top of the lid is between 8.0 and 9.5 km s⁻¹, below which a constant velocity (7.25–7.75 km s⁻¹) half space exists. A thin high density garnet layer would be stable at this depth in the lunar interior⁶.

With the exception of the first 8 km of the Moon and the mantle velocity, the range of our solution spans published solutions which have used both travel times and amplitudes. We used a one parameter model for the near surface structure. Had we used a more complex model our solution would also have spanned the published solutions in the near surface layers. We feel that detail in the near surface layer is an unjustified complication. The discrepancy below 1,665 km is resolved if head waves and tunnelled waves exist in a high velocity lid.

We thank G. V. Latham for making available the data in Table 1, and M. N. Toksoz for the results in Table 2.

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Received June 7; revised November 17, 1974.

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Short period teleseismic S waves

SHORT range observations of short period S waves are useful in determining the distribution of zones of high and low anelastic attenuation in the vicinity of island arcs¹⁻³. We show here that S waves are also recorded at some stations at teleseismic ranges and that these recordings can be used to study anelastic attenuation.

S waves are useful because they are more sensitive to changes in anelasticity than P waves. The attenuation of body waves at angular frequency ω is given by $\exp(-\omega t^*/2)$ where $t^* = T/Q_{av}$. T is the travel time and Q_{av} the average quality factor Q , for the raypath and the particular wave type. As S wave travel times are about $\sqrt{3}$ of the travel times of P waves, and Q_β is about $4/9$ Q_α (making the usual assumptions that anelasticity affects only the shear modulus of rocks^{4,5} and that Poissons ratio is around 0.25), t^* for P waves (t^*_α) is roughly a quarter of t^* for S waves (t^*_β). Thus, for an S wave travel

Table 1 Crustal model for receiver crust

	P wave velocity (km s ⁻¹)	S wave velocity (km s ⁻¹)	Density (g cm ⁻³)	Thickness (km)
1st layer	5.6	3.2	2.8	12.0
2nd layer	5.9	3.4	2.8	12.0
3rd layer	6.2	3.6	3.2	14.0
4th layer	8.3	4.8	3.4	∞

time of 1,000 s an increase in anelasticity that produces an increase in Q^{-1}_β of 0.001 gives, at 1 Hz, 10 times more attenuation of S waves than of P waves. Short period S waves are difficult to detect at teleseismic distances because for many paths through the Earth t^*_α is apparently about 1.0 s or greater^{5,6,7} so that t^*_β will be 4.0 s or more. Thus (at 1 Hz), S waves will be about 10,000 times smaller than P waves, at the receiver, other things being equal.

Values of t^*_α in the range 0.2–0.5 s have, however, been reported^{8,9} on some paths, suggesting that differences between the attenuation of P and S waves for these paths should be in the range 10–100. Short period S waves should, therefore, be detectable at long ranges. A search of recordings from array stations Yellowknife, Canada (YKA) and Warramunga, Australia (WRA) shows that both stations do sometimes record short period S waves from earthquakes with (US Coast and Geodetic Survey) body wave magnitudes greater than 4.8. The largest S waves relative to P waves are from deep earthquakes. As most attenuation is thought to occur in the asthenosphere at depths in the Earth of 100–300 km, and the

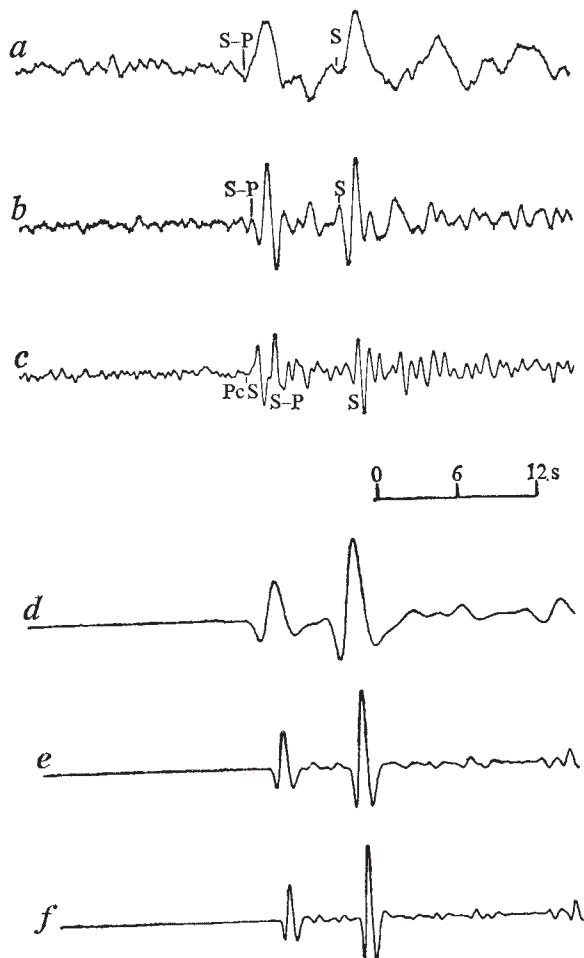


Fig. 1 Observed (a-c) and modelled (d-f) S wave seismograms showing double arrivals. The later arrivals on the observed seismograms are interpreted as true S waves and the preceding arrivals as S to P conversions at the Moho beneath the receiver. The modelled seismograms include no effects of layering in the source region; all the arrivals are generated from a single S wave arrival at the base of the receiver crust. Note that the PcS wave for earthquakes at the depth and distance illustrated in c (P wave reflected from the core as an S wave) arrives just before the S-P arrival. a: November 2, 1972, Loyalty Islands; origin time 19 h 55 min. 22.1 s; $m_b = 6.3$; $\Delta = 32.40^\circ$; $h = 32$ km. b: October 26, 1972, New Hebrides Islands; origin time 22 h 48 min. 34.4 s; $m_b = 5.4$; $\Delta = 31.9^\circ$; $h = 157$ km. c: March 9, 1971, south of the Fiji Islands; origin time 08 h 11 min. 52.8 s; $m_b = 5.0$; $\Delta = 42.4^\circ$; $h = 511$ km. d, $t^*_\beta = 3.5$; e, $t^*_\beta = 1.3$; f, $t^*_\beta = 0.7$.

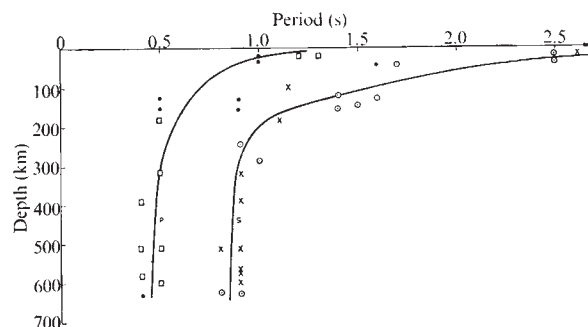


Fig. 2 Dominant period of direct P and S waves observed at WRA as a function of the depth of focus of the source; combined data from Tonga-Fiji ($\square$, $\times$) and New Hebrides ($\bullet$, $\circ$) regions.

asthenosphere is thought to thin under shields, then observations of large S waves from deep earthquakes at stations such as YKA and WRA, which are situated on shields are just what is expected (compare Fig. 1a and c).

The largest number of S waves that we have obtained are from WRA for the Tonga-Fiji and New Hebrides regions, and as these are the regions where Q has been most extensively studied at short ranges, we shall concentrate on these observations.

Figure 1 (a, b and c) shows WRA recordings of SV waves (the array contains only vertical component seismometers) from three earthquakes. All the examples show two distinct arrivals separated by 6 s; both have apparent surface velocities close to those predicted by S wave travel-time tables. The second of the two pulses seems to be a true S wave; the first is interpreted as a P arrival generated by S to P conversions at the base of the crust at WRA. Explanations such as double sources can be ruled out because the P waves show no similar double arrivals; and the possibility that the second pulse is a depth phase can be discarded because the double pulse is generated by earthquakes at all depths with a constant separation of about 6 s.

The existence of P precursors to SV have been suspected for many years. Jeffreys¹⁰, for example, proposed them as an explanation of the difficulties that occurred in reconciling P and S travel times, for it seemed that S waves from many earthquakes arrived earlier than predicted.

The double pulse can be modelled theoretically (Fig. 1d, e and f) using the method described by Hudson^{11,12}; the WRA crustal structure used (Table 1) is based on a model¹³ for the Canadian Shield. Kanesewich¹⁴ *et al.*, have made similar theoretical calculations and discussed the implications of precursors to S waves, and Båth and Stefánsson¹⁵ have observed precursors to S waves at teleseismic distances on long period instruments. The examples shown here seem however, to be the first short period observations of such precursors at these distances, although Smith¹⁶ has observed similar effects from local earthquakes.

To ascertain the effects of attenuation we measured the dominant period of the P and S waves as a function of the depth of focus (see Fig. 2 and ref. 2). As there are no obvious differences between the data from Tonga-Fiji and those from the New Hebrides they are both shown on the same figure. The results show an increase in the period of both the P and the S waves with decreasing depth of focus for depths shallower than 250 km but, as expected, the increase in period is much more pronounced for S waves than for P waves. Assuming that these differences in period can all be attributed to the effects of anelastic attenuation it is possible to deduce a rough *Q* structure for the upper mantle in the Tonga-Fiji and New Hebrides regions (Table 2). The structure was obtained by modelling P and S recordings for a range of values of *t** and measuring the predominant period of the resulting seismograms. Using this relationship between *t** and period, we estimated the value of *t** for paths to WRA for each depth of focus (using the smoothed curves drawn through the data, Fig. 2). The upper mantle was then divided up into a series of layers in which *Q* is assumed constant, and the *Q* structure was derived using the time spent in each layer by rays travelling to WRA for each depth of focus.

The result shows very low *Q* values at depths shallower than about 200 km in both the New Hebrides and Tonga-Fiji regions. The low *Q* in the Tonga-Fiji region seems to coincide with the zone of "extremely low *Q*"² beneath the Lau Basin west of the Tongan Ridge, although we shall have to use ray tracing to work out the details of structure. The similar zone of extremely low *Q* lying to the south and west of the New Hebrides region does not seem to have been reported previously.

Table 2 Approximate *Q* structure for the upper mantle

Depth (km)	<i>Q</i> ⁻¹ _a	<i>Q</i> _a	<i>Q</i> ⁻¹ _b	<i>Q</i> _b
0-100	0.0250	40	0.0500	20
100-225	0.0110	90	0.0220	45
225-600	0.0010	1,000	0.0033	300
600	0.0004	2,500	0.0008	1,250

In deriving the *Q* structure we have made the assumption that any source effects on the observed period can be neglected. This is probably an oversimplification; it may be that for shallow sources the pulses radiated are of longer duration and, therefore, the ratio between high frequency energy and low frequency energy is lower than for deeper sources. We have, however, found it impossible to model seismograms showing S waves with a period of over 2 s by increasing the source size while obtaining a good fit to the P phase at the same time. For example, PKiKP is visible for the Loyalty Islands earthquake (although not illustrated in Fig. 1a) as a single wavelet with a period of 0.8 s, and the S phase for this earthquake has

a period of 2.5 s. With the source model used here these differences in period cannot be shown as an effect of source rather than of attenuation.

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Possible mechanism of continental breakup in the North Atlantic

NEW observations from the Skaergård area have provided evidence relating to the mechanism of continental breakup in the North Atlantic. The East Greenland coastal flexure and its associated dyke swarm¹ has become a classic of this type of structure and has been frequently compared with similar structures around the world, notably the Lebombo monocline of south-east Africa^{2,3}. No new data have appeared in print, however, since the original description by Wager and Deer¹. I have had the opportunity to examine the structures in the Skaergård area (68°N, 32°W), and report here my observations on the mechanism of flexuring and dyke intrusion, which is believed to be very similar to structures described from areas of present spreading, such as Afar⁴.

In the original description Wager and Deer emphasised that the coast parallel dykes were intruded radially to the flexuring from a centre below surface, caused by tension resulting from the bending of a thick succession of lavas (Fig. 1a). Closer examination of a 500-m profile at nearly right angles to the coast at Haengefjeldet, south of Skaergård (Fig. 2), has shown that the continuous decrease in dip on approaching the coast, described by Wager and Deer is a severe over-simplification. Most of the dykes in the swarm have average dips of 90-70° N and strike 90-100°, an orientation which seems to be rather constant from the coast and several kilometres inland. All dykes have very irregular dips and several are found which have dips varying from northerly to southerly directions. Examination of the dyke chills in the walls showed that dilatation is the normal intrusive mechanism and that the host rock in

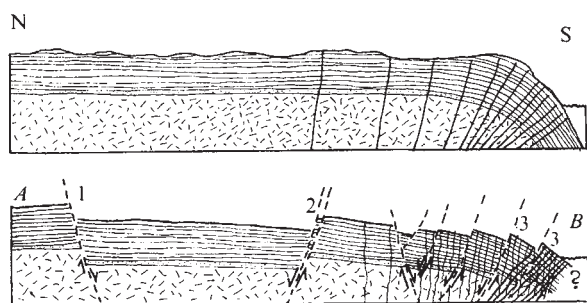


Fig. 1 Sketches of the profile A-B (Fig. 2) showing (upper) the model proposed by Wager and Deer¹ compared with (lower) that proposed in this paper. The older generation of dykes is shown in heavy black, the younger as the thinner, approximately vertical dykes. Numbered faults correspond to those shown in Fig. 2.

several places is an older dyke swarm of thick, black, coarse-grained dykes.

This older dyke swarm which is also parallel to the coast, is very uniform in the field and in hand specimen and has low dips of 50–40° N. It cuts the lavas, agglomerates and tuffs of the lower basalt series⁵, which are orientated 100/45–60° S and so is perpendicular to these. Both lavas and older dykes are cut by numerous small shear zones and faults, now filled with carbonate and zeolites. The vesicles of the lavas are also completely filled. The mineralogy of the older dykes and the lavas has suffered from this altera-

tion such that olivine always is replaced by chlorite, serpentine, carbonate, and so on, while plagioclase is less altered and pyroxene is always fresh. The large amount of pseudomorphed olivine both in the groundmass and as phenocrysts, suggests that the older dykes were very basic olivine basalts. Chemical work on this subject is in progress and will be presented elsewhere.

In contrast, the younger main swarm with grey, green, brown and red weathering colours shows no signs of shearing and faulting and is much less altered. As suggested by the weathering colours the younger swarm is characterised by a wide spectrum of different rock types, including both aphyric and plagioclase-aphyric dolerites together with a range of intermediate to acid types. The distribution of dykes with a concentration near the coast described by Wager and Deer¹ and confirmed by my own observations, was regarded by them as being the consequence of the maximum bending of the coastal flexure in this area (Fig. 1a). It now seems, however, that the high intensity of dykes near the coast is caused by the presence of both dyke generations within 1 km from the coast, whereas only the younger generation persists further inland, at least up to 5 km, with little or no decrease in intensity.

These observations clearly show that the younger dykes were not intruded perpendicular to the lavas and that they were intruded after the tilting of the lavas. The older dykes, which, in common with the lavas, are sheared and altered, must have been formed before the flexuring, and thus neither of the generations can have formed as the result of the process described by Wager and Deer.

The faulting and shearing described in the profile at Haengefjeldet has been found in the whole of the Skaergård area, and the profile A-B (Fig. 2) will now be described in some detail. In the description of the Skaergård area, Wager and Deer⁶ noted the Sorte Kap fault 60 km inland as a normal fault with downthrow to the south and a dip-slip of at least 800 m. North of this fault the lavas dip slightly north and to the south dips are small but southerly (Fig. 1b). At the lake in Sødalen, Mikis Fjord, a normal fault was located running E-W and dipping north with a downthrow to the north and a dip-slip of several hundred metres, shown by the fact that the underlying sediments are relatively up-thrown and exposed to the south. North of this fault the lavas dip 10° S while to the south of it the dips have increased to 20–22° S.

Although minor faults occur, dips of the lavas are more or less constant as far as 5 km from the coast along the Denmark Strait. Because of the intensity of the younger generation of dykes in the dyke-swarm along the coast, not disturbed by faults, outcrops of the lava succession are difficult to correlate, but within the last 5 km to the coast, dips change from 22° S to 45–60° S. It is believed that this change occurs principally at two or more major shear zones, which have been seen in the outer Mikis Fjord and at Haengefjeldet.

Shear zones have been found to be very common in the coastal region but at most localities displacements are very difficult to evaluate, because of the lack of marker horizons. For example six shear zones or faults cut the Skaergård peninsula, but in only one of these could the displacement be confirmed by a dyke intersection.

These relationships in Mikis Fjord area are very similar to those described by Mohr⁴ from the western escarpment of the Afar triangle in Ethiopia. The model proposed for the East Greenland coastal flexure, is that of a similar large scale fault pattern with marginal normal faults dipping S (such as the Sorte Kap fault) and a large number of smaller northerly dipping faults near the coast with downthrow to the north, causing a rotation of fault blocks and the dip variations in the lavas.

This interpretation corresponds to a 'half' graben structure, which is consistent with the plate-tectonic origin of

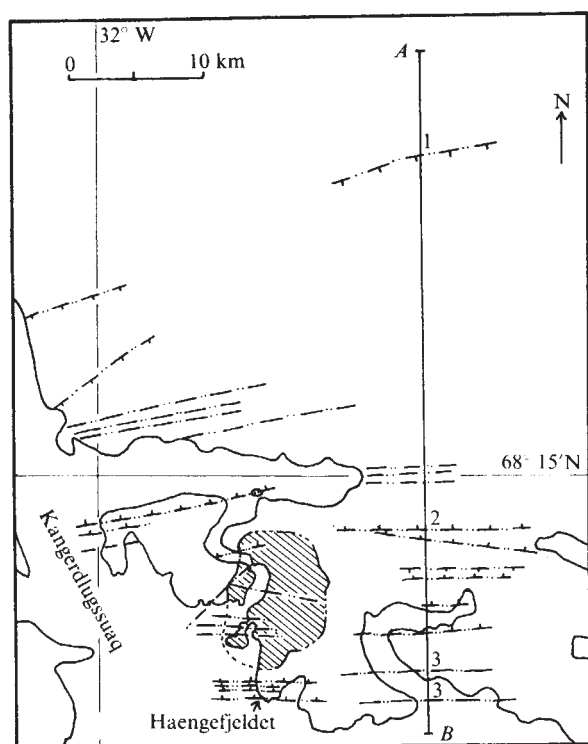


Fig. 2 Simplified map of the Skaergård area, East Greenland. The Skaergård intrusion (shaded) and faults with dip directions are shown. Faults without dip signs are shear zones where the faultplane is more or less vertical and relative displacement has not been determined. 1, Sorte Kap fault; 2, Sødalen fault; 3, Mikis Fjord major shear zones (Fig. 1b).

the lower Tertiary geology of this area. Thus, Brooks⁷ described the Kangerdlugssuaq areas as the site of the Icelandic mantle plume in Eocene times, which resulted in major updoming, rifting about a triple point and subsequent crustal separation along fractures parallel to the present coastline.

As it is assumed that both dyke-swarms and the flexuring of the coast are formed in the breaking up of the North Atlantic, the correlation between dyke intrusion and major tectonic events might be explained in the following manner. As stated earlier, both the older dykes and the lavas are flexured and therefore must be older than the collapse of the coast marked by the faulting and shearing. Thus these dykes may well be correlated with the prespreading updoming described by Wager and Deer⁵ and Brooks⁷.

The younger dykes, which are not sheared and faulted, were probably formed after the initial fracturing event and could be correlated with the active spreading and new crust formation, which opened up the North Atlantic.

Finally, the recognition of intense faulting in the area indicates that extreme caution must be exercised when employing the structural heights in the Skaergård intrusion as estimated by Wager and Brown⁸. Tectonic disturbances of the intrusion would explain some of the irregularities of the Skaergård contact, which can be seen on aerial photographs and the map of Wager and Brown⁸. Such faulting would also be expected to have a profound effect on the magnitude of the hidden layered series in the intrusion, whose size has recently been questioned by geophysical⁹ and geochemical¹⁰ investigations and on estimation of the thickness of the basalt succession in the area.

I thank Professor A. R. McBirney for placing aerial photographs of the area at my disposal. Dr C. K. Brooks, Mr T. Haaland and Mr T. S. Petersen helped in the field and in the writing of this report.

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Interaction between coherent light waves and free electrons with a reflection grating

A NEW effect is proposed for obtaining effective interaction between coherent light waves and free electrons. Potential applications of the effect are optical electron accelerators (linac)^{1,2} and optical amplifiers³.

Smith and Purcell demonstrated⁴ that light is emitted when a high voltage electron beam moves parallel and close to a metallic optical diffraction grating in a direction perpendicular to the grating rulings (Fig. 1a). The radiation has been explained physically in terms of the oscillations that must be executed by the charge induced on the grating surface by an electron in the beam. The dispersion relationship is

$$\cos \theta_n = (c/v) - (n\lambda/d) \quad (1)$$

with $n = 1, 2, \dots$, θ_n the angle that the direction of propagation

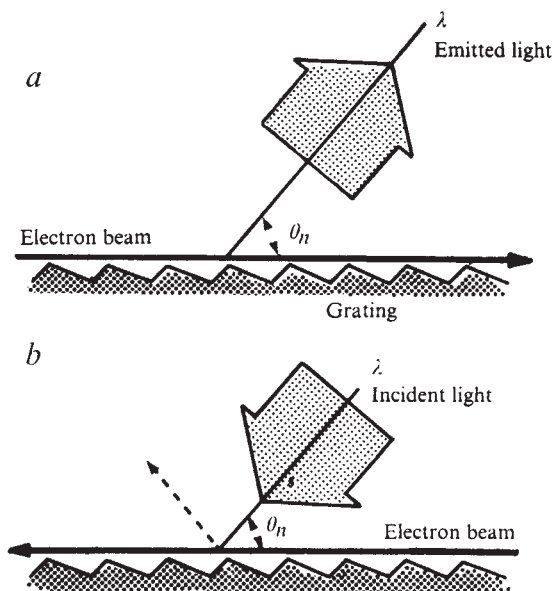


Fig. 1 a, Schematics of the Smith-Purcell radiation; b, 'inverse Smith-Purcell effect'.

makes with that of the electron beam, d the grating constant, v the velocity of the beam, and λ the free space wavelength of the light.

Here we consider the 'inverse Smith-Purcell effect' to obtain extended interaction of an electron beam moving along the grating surface with light incident on the surface. A simple Huygens analysis shows that when the directions of the incident light wave and the electron beam are opposite to those in the case of Fig. 1a and satisfy the condition of equation (1), the beam interacts synchronously with the wave; that is, an electron sees the same phase of the wave with the pitch of d (Fig. 1b). When the electron beam is opposite in direction to the wave on the grating surface, the synchronous condition becomes

$$\cos \theta_m = (m\lambda/d) - (c/v) \quad (2)$$

where $m = 1, 2, \dots$

When the synchronous condition is satisfied, an effective interaction must occur between the electron beam and the light wave in the same manner as the spacial harmonic interaction occurring in microwave travelling-wave tubes, leading to

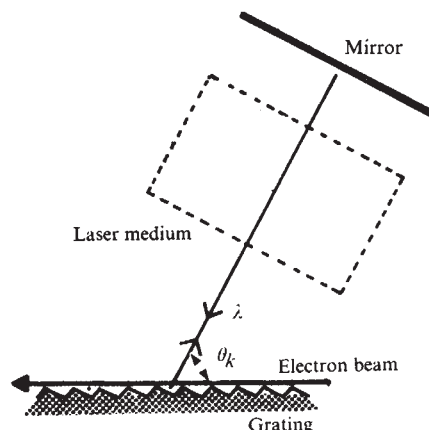


Fig. 2 The Smith-Purcell and 'inverse Smith-Purcell' arrangements with a Fabry-Perot resonator.

electron acceleration or deceleration according to the relative velocities of the electron and the phase of the light wave in the interaction region. If the electron suffers net acceleration over a period of time, then electromagnetic field energy is converted into electron kinetic energy, resulting in an electron accelerator. Optical (laser) accelerators will have the unique property of providing high acceleration gradients because of the intense fields that can be obtained. An acceleration of the order of 1 GeV m^{-1} may be anticipated³. In addition, the electrons will be bunched to a dimension which is short compared to the light wavelength, giving current pulses with a time duration of the order of $\sim 10^{-15} \text{ s}$. These pulses could be used to study ultra-fast relaxation processes^{1,3}.

On the other hand, when the electrons are faster than the phase velocity of the light wave and suffer a net deceleration, light amplification may be obtained.

If the incident angle θ_k of the light satisfies the following condition,

$$\cos \theta_k = k\lambda/2d \quad (3)$$

where $k = 1, 2, \dots$, the diffracted ray direction is the same as the incident one. In this case we can adopt the configuration shown in Fig. 2. A Fabry-Perot resonator is formed with the grating and a mirror⁵. Using this configuration as a laser cavity, high intensity electric field in the laser cavity can be used to accelerate the electrons. This configuration involves the one proposed by Takeda and Matsui² as the special case of $\theta_k = \pi/2$.

The same configuration can be used to obtain 'stimulated Smith-Purcell radiation', that is, the Smith-Purcell radiation with internal feedback by the Fabry-Perot resonator. We have observed⁶ the millimetre and submillimetre wave oscillation in an electron tube called the Ledatron when $\theta_k = \pi/2$.

With recent progress in the development of high power lasers in the optical and infrared regions, it should be possible to miniaturise linacs by using the 'inverse Smith-Purcell effect' proposed here.

We thank Professor R. H. Pantell of Stanford University for communicating unpublished information.

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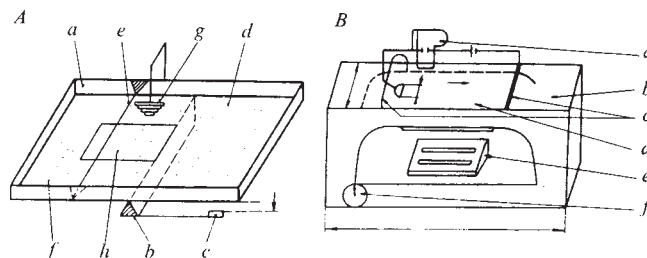


Fig. 1 Experimental apparatus. A: a, Tub; b, wedge; c, washer; d, base; e, trip-wire; f, fine sand; g, camera; h, section. B: a, Camera; b, small channel; c, electrodes; d, glass plate; e, stroboscope; f, pump.

Marked boundary layers exhibit a clearly defined reproducible pattern of streamwise streaks in their sublayers¹⁻³ and regions with higher colour concentrations are identical to those areas where liquid flow is retarded.

Observations by Fales⁴ allowed this flow structure to be explained by means of a longitudinal eddy model. As the question of this eddy structure, or its alteration in the existing models is of decisive importance^{2,5,6} in explaining the Toms effect we endeavoured to explain this problem by observation.

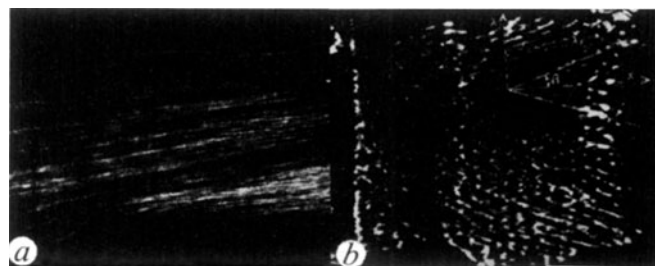


Fig. 2 Sand pattern experiments. a, With tap water; b, with 50 p.p.m. polyacrylamide solution.

We investigated the structures using fine sand to make the flow visible, though in fact it is not the flow itself which is observed, but the trace it leaves on the bed. As a second step we investigated the sublayer by means of the hydrogen bubbles visualisation technique.

Fine quartz sand settles evenly on the bottom of the experimental tank, and when the liquid is set in motion, the individual grains can be photographed easily, particularly when a smooth surface is used, as in this case. When we investigated integral structures, we used continuous illumination; short term movements were followed under stroboscopic lighting.

As a test device (Fig. 1a) we chose a shallow, rectangular sheetmetal trough with a smooth, black plastic foil attached to the bottom. The trough could be tipped steeply about a pivot by means of exchangeable washers, so that we could regulate the velocity of flow as a result of the tipping motion. The flow itself was also brought to a turbulent state with a trip-wire and made visible by means of fine sand. We recorded the movement of the quartz grains with a camera mounted perpendicular to the water surface. The second test was carried out in a small closed-circuit channel (Fig. 1b). The camera, or film unit, stood perpendicular to the two-dimensional flow, which was illuminated from below by a stroboscope through a glass plate.

Alteration of structures of sublayer flow in dilute polymer solutions

THE Toms effect may be assumed to be based on a phenomenon which occurs in the immediate wall layer. Models proposed to explain the effect are, therefore, mostly aimed either at producing an interaction of the molecules with the flow structure (that is, the eddies), or at attributing new rheological properties to the liquid on the basis of the molecules released, which should lead to new forms of flow.

The anode was a stretched wire 8 cm long, placed transverse to the flow and as close to the wall as possible in order to produce the bubbles in the sublayer. The cathode was used as a spillway in order to set the water depth to approximately 2 cm. The mean velocity of flow was 10 cm s^{-1} , corresponding to a Reynolds number of 2,000. As the sand tests were performed at the same Reynolds number, it was possible to compare the two results. The greatest problem, however, was the 100 p.p.m. MEYP-01 polyacrylamide solution used, because the hydrogen bubbles contributed considerably towards the destruction of the molecules and the test liquid had to be replaced with freshly prepared solution relatively quickly. For quantitative tests, therefore, the closed circuit was dispensed with, as in this case it is only permissible to work with fresh solution, which would entail substantial experimental difficulties, particularly when high demands were made of the solution's homogeneity.

But it is not only the bubbles which can alter considerably the liquid; the molecules also contribute towards distortion of the bubble pattern as a result of pollution of the anode. We took that into account with frequent cleaning of the wires. As this resulted in a conflict between an undisturbed flow pattern and flow disturbed by the cleaning, we took care to work under optimum conditions. The flow patterns were both photographed and filmed. The film was shot with a speed of 24 or 32 frames s^{-1} .

For pure water, we obtained structures which have already been observed and described¹⁻³ (Figs 2a and 3a). Polyacrylamide solutions (50 p.p.m.) produced a considerably modified sand pattern compared with that produced by pure water, distinguished particularly by the formation of dunes (Fig. 2b). As the interaction between polymer solutions and quartz particles is still not known, it is difficult to explain this phenomenon by one of the various models.

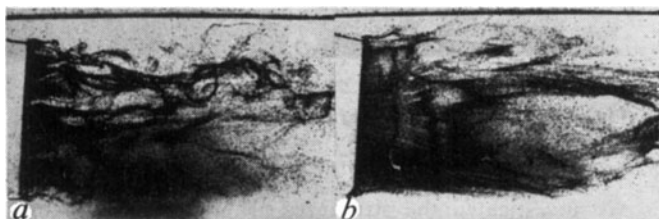


Fig. 3 Hydrogen bubble experiment. a, With tap water; b, with 100 p.p.m. polyacrylamide solution.

This observation suggests, however, that in the turbulent boundary layer of a dilute polymer solution, longitudinal eddy structures were suppressed or deflected from the direction of flow in the immediate vicinity of the bed of a smooth wall.

In the second experiment we again found a thickening of the sublayer in our tests with polymer solutions. It was clearly a transverse eddy formation (Fig. 3b) which dominated the picture.

Although the films would permit some degree of quantitative evaluation the test set-up was not designed so that figures obtained from it would be generally valid. Nevertheless, there is no doubt that the change already perceived visually may be considered as qualitatively established.

It is worth noting that Brennen⁷ observed a transverse wave structure for flow separation at spheres in the case of thin polymer solutions, which points to a similarity of the two phenomena.

Rudd⁸ provided more direct evidence of this change and demonstrated, with laser doppler measurements near the wall, a significant increase in the variation of the velocity components in the direction of flow, whereas practically no difference from pure water could be determined along the pipe axis. Exactly the opposite behaviour was found for velocity fluctuations transverse to the direction of flow; they decreased in comparison with pure water near the wall, but increased in the outer region.

This fact is immediately evident when the variations in the sublayer are derived from eddies moving past, which in the case of the polymer solutions are deflected to a greater extent from their original position in the direction of flow ($u'=0$) into the direction at right angles ($w'=0$).

Similar arguments come from local correlation measurements which, on the basis of their chronological sequence, represent a measure of the frequency of occurrence of a certain eddy structure. Thus, a considerable increase in the degree of correlation is found in the direction transverse to the flow, with a far slower decay of this correlation in the direction of flow⁹.

The appearance of a larger number of transverse eddies is an almost ideal explanation of these measurements. In the direction at right angles to the flow, the movement is strictly influenced by the eddy itself. As a result of the high stability of the eddy structures during transportation by the flow the influence lasts longer⁹.

The thickening of the sublayer also leads to an enlargement of the individual eddy diameter, so that the fundamental change of the flow structure in the sublayer varies from thin longitudinal eddies in pure water to relatively thick transverse eddies in dilute polymer solutions.

To return once more to our work with fine sand, we now understand how a dune structure occurs transverse to the direction of flow. In principle the same as the longitudinal eddy structure, but it is wider because of the thicker sublayer.

This, however, only explains the formation of dunes, not their marked wedge shape, which must be the result of a locally varying transfer process. As the dune bank formed transverse to the flow is eroded at the point where the flow in this sublayer exhibits higher Reynolds stresses, it is natural to consider the sand pattern as a dune formation which is eroded locally by shearing waves.

The pattern itself has a self-stabilising effect, as the shearing waves are channelled by the topography of the sand. The stochastic disturbances which possibly still occur locally and sporadically, and which spread out as shearing waves, are thereby fixed; this results from an interaction with the mobile bed. So no comment can be made about the local stability of the disturbances; only about the relationships between wavelengths in both directions of propagation. This relationship determines the mean direction of propagation of the disturbances and can be found from the characteristic apex angle β . Assuming that these disturbance waves appear in the wedge structure β can be measured (Fig. 2b). For our test, it averaged $14.5 \pm 1.5^\circ$.

Morrison¹⁰ *et al.* specified a disturbance wave model for sublayers and took measurements by frequency wave number spectra on turbulent flow in pipes.

Using shearing velocity and kinematic viscosity as scaling values, they found, expressed dimensionless in these magnitudes, well-defined wavelengths transverse to the flow and very weakly defined wavelengths in the direction of flow. The characteristic angle was 12° .

The sublayer is thickened substantially by the addition of polymers and the disturbances are propagated in the form of shearing waves, which do not differ greatly from those in pure water.

This alteration in structure, as can be demonstrated clearly, indicates that it would be premature to draw speculative conclusions at this stage about the Toms effect itself.

Rather, it needs to be explained why the concentration of vorticity is rotated.

As one of the most obvious explanations lies in associating this behaviour with normal stresses, this indicates an elastic effect.

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Received August 15; revised September 30, 1974.

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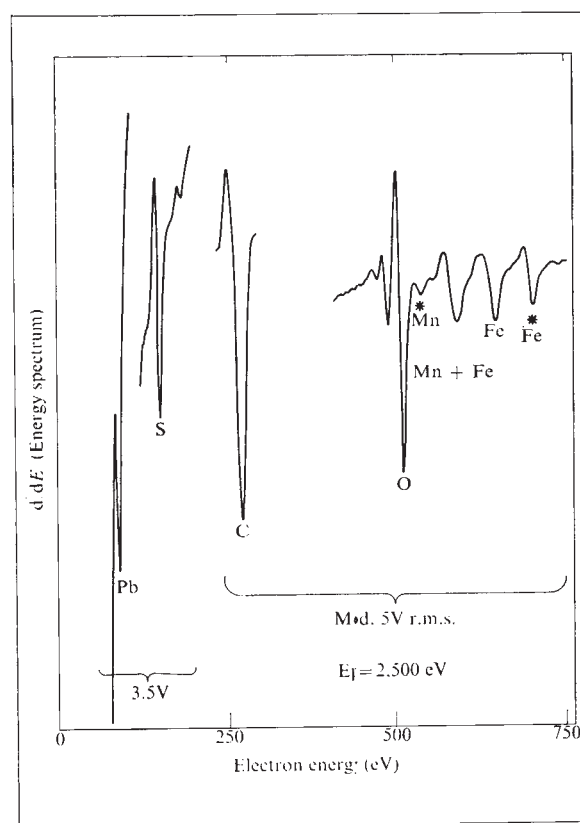


Fig. 1 Auger electron spectrum of the surface of leaded free-machining steel swarf. The swarf surface had contacted the rake face of the tool during machining at a surface speed of 120 feet min^{-1} (0.61 m s^{-1}). The steel had the following composition: C, 0.10%; Si, 0.01%; Mn, 1.0%; S, 0.30%; Pb, 0.18%; Ni, 0.03%; Cr, 0.01%; Mo, 0.01%; Cu, 0.10%; P, 0.063%; N, 0.004%. The ordinate is a derivative of the electron energy distribution, $n(E)$.

Lead monolayer lubrication in steel machining studied by Auger electron spectroscopy

ALTHOUGH free-machining steels are widely used in the manufacturing industry because of improved machining characteristics, such as prolonged tool life, the detailed mechanisms by which the additions of lead and manganese sulphide bring this about are subject to debate. We report the existence, on the swarf from leaded free-machining steel, of near monatomic layers of lead which are thought to enhance the lubrication of the swarf-tool interface.

The steel was dry machined at six cutting speeds in the range of 50–350 feet min^{-1} (0.25 – 1.78 m s^{-1}) using a new M2 high-speed steel cutting tool for each speed. The cutting tool geometry conformed to the relevant International Standards Organisation recommendation. Samples of the swarf were carefully collected to avoid contamination and were then analysed by Auger electron spectroscopy (AES).

AES is an electron probe technique which gives an elemental analysis of the few uppermost atom layers at a surface. Combined with ion beam etching, AES provides element composition profiles of the surface layers with in-depth resolutions as good as 1 nm. Figure 1 shows a typical spectrum of a swarf surface (air-exposed) which had contacted the rake face of the tool, indicating a very strong concentration of lead and manganese sulphide in the surface layers of the swarf compared with the bulk. The uniform distribution of the lead over the swarf surface is illustrated in the 'Augergraph' in Fig. 2. The lead Auger electron signal has been used as a means of recording the distribution of lead; this resembles the use of X rays in the conventional electron microprobe but here refers more critically to the one-or-two atomic layers nearest the surface. Similar spectra to that in Fig. 1 were obtained from the other side of the swarf which had previously contacted the flank face of the tool.

Further information on the composition of the surface layers of the swarf was obtained by ion beam etching. Figure 3 shows that both carbon and lead were largely removed from the surface in the period of the ion bombardment, whereas manganese remained. The decrease in sulphur concentration is an artefact, attributed to oxidation of manganese sulphide induced by electron beam irradiation in the presence of residual water vapour in the Auger electron spectrometer. This was checked by moving the electron beam to a neighbouring position on the etched surface, thus restoring the sulphur signal to its original value. The ion bombardment was continued

for a total of 1,680 s resulting in further removal of lead, the manganese concentration remaining constant. The profile shows that the surface region of the swarf consists of a series of layers, an outer contamination layer containing carbon, then an extremely thin layer of lead which in turn covers a composite substrate of iron and relatively thick patches of manganese sulphide.

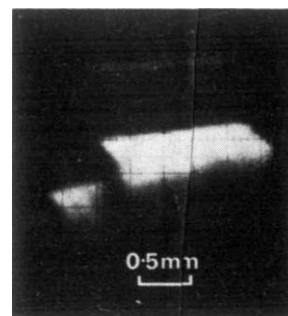


Fig. 2 Scanning electron Auger micrograph (Augergraph) of leaded steel swarf showing uniform distribution of lead (bright areas) on the swarf surface. This structure was obtained by programming the primary electron beam ($50 \mu\text{m}$ diameter) to describe a raster on the target (as with the scanning electron microscope) and by using the signal from the lead Auger electron peak to modulate the brightness of a video display¹.

The ratio of the observed lead (91 eV) to iron (700 eV) Auger electron peak-to-peak amplitudes ranged from 1.5 to 8.5 for swarfs obtained at cutting speeds between 50 and 350 feet min⁻¹ (0.25 and 1.78 m s⁻¹). The variation in the ratio with cutting speed is the subject of a further investigation. The expected relative peak intensity value for one monolayer of lead on iron is 9.2, calculated from the Auger electron spectra of the pure elements and from measured electron ranges in similar elements^{2,3}. It seems therefore that the lead is concentrated in a layer about one monolayer thick. Further evidence for this localisation is obtained from the shape of the sputtering profile. The predicted profile from sputtering statistics for one monolayer or fraction of a monolayer of lead on iron is of the form $\exp(-nyt)$ where n is the number of argon ions per target atom per second, y is the sputtering yield and t is time¹. Figure 4 shows the observed exponential decrease with ion bombardment time.

To obtain the precise thickness of the lead layer from the profile it is necessary to know the relevant sputtering yield. The appropriate sputtering yield in this case is that for lead from the surface of iron, both elements having been oxidised by air exposure; this yield is not known. It may be expected to differ from the value for bulk lead since Tarng and Wehner⁴ have shown that the sputtering yield of an atom is strongly dependent on the substrate. From the ion beam current density ($n=1.2 \times 10^{-2}$ ions per lead atom per second) a value of $y=0.35$ would be consistent with a monolayer lead level. As expected, this value is lower than the yield for bulk lead since yields are

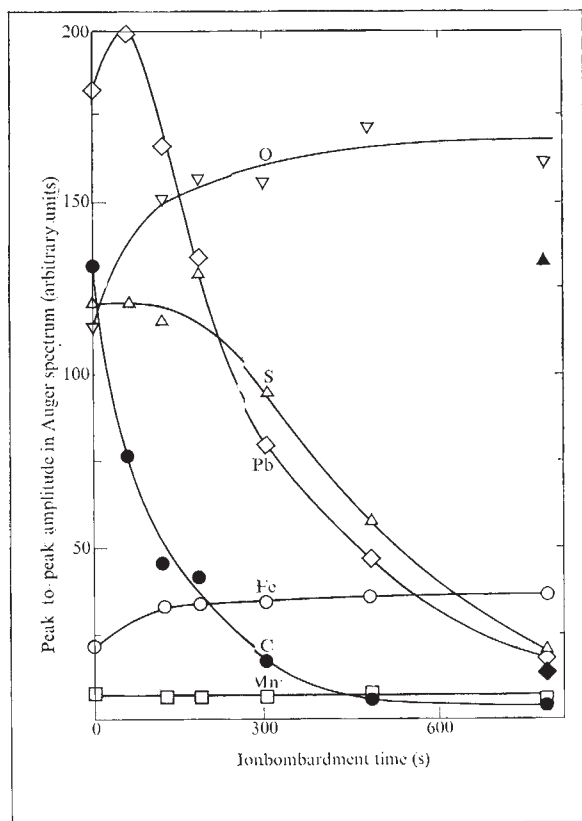


Fig. 3 Element concentrations normal to the surface of the swarf described in Fig. 1. The profile was obtained by sputtering at normal incidence with 620 V argon ions and monitoring with AES. The Auger electron peaks used were those shown in Fig. 1; in the case of iron and manganese the peaks used are marked with an asterisk. The filled symbols ($\blacktriangle$ and $\blacklozenge$) on the right of the figure refer to sulphur and lead after moving the electron beam to a neighbouring position on the ion etched surface (see text).

inversely proportional to heats of sublimation⁵, and surface energy measurements indicate that lead adsorbs strongly on the surface of iron⁶.

It is considered that the lead layer is formed on the surface of the swarf during cutting by a process of extrusion due to the high compressive forces in the primary deformation zone. The movement of lead would also be assisted by the high local temperatures generated during cutting. It is important to note that not more than one monolayer of lead is formed on the swarf over the range of cutting speeds investigated. This may be due to the higher binding energy of the first layer of lead atoms to the iron substrate, compared with the second layer of lead atoms to the first.

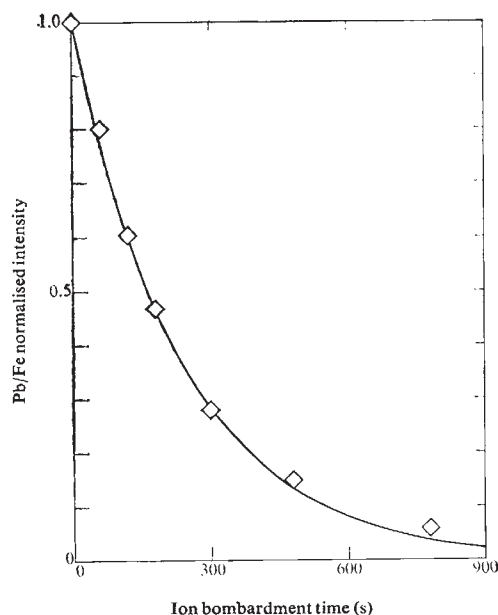


Fig. 4 Sputter profile normal to the swarf surface for lead relative to iron derived from Fig. 3. The curve is the exponential fit described in the text.

The wear mechanisms relevant to cutting tool wear and tool failure have been reviewed by Tabor⁷, the operative wear mechanisms depending critically on temperature and the properties of tool and workpiece. Wear processes of M2 high speed steel during the machining of a wide range of free-machining steels have recently been studied (S. Akhtar and B. M., unpublished), confirming that the presence of lead in resulphurised free-machining steels gives an additional improvement in cutting tool life above that conferred by manganese sulphide alone. Their study has also confirmed that the principal wear mechanisms in the cutting range 50 to 325 feet min⁻¹ (0.25 and 1.65 m s⁻¹), using high speed steel cutting tools, are adhesive and abrasive wear. It would seem that a layer of lead on the swarf at this surprisingly low monolayer level is sufficient lubricant to give improved tool life by the reduction of adhesive wear. It is known that the equilibrium segregation of a minority species to monolayer levels at grain boundaries leads to reduced grain boundary cohesion⁸ and that lead can induce brittle failure of high strength steels⁹. Similarities may exist in the present reduced adhesion between the swarf from leaded free-machining steel and the tool.

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Received October 31; revised November 29, 1974.

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Lattice resolution in an electron-beam sensitive polymer

RECENT developments in our understanding and application of high-resolution defocusing phase contrast electron microscopy have contributed significantly to structural studies of carbon fibres from a range of precursor materials^{1–4}. For example, we are now able to follow the natural pattern of carbonisation in the standard commercial process for polyacrylonitrile (PAN)-based carbon fibres, and to detect variations in structure which give rise to abnormal physical properties^{5,6}. One major factor which has influenced the success of the work on carbon fibres is the stability of the stacked carbon ribbons to irradiation by an electron beam, and the relative ease with which the {002} lattice fringes can be recorded. In fibrous organic polymers we have observed massive degradation of structural order as revealed by the rapid deterioration of the electron-diffraction pattern^{7,8}; this has precluded the recording of lattice-fringe images and so prevented the breakthrough in understanding which would undoubtedly follow as a result of direct observation of fibre structure at the molecular level.

Here we report for the first time the direct imaging of crystallite lattice fringes in a beam-sensitive polymer together with a preliminary analysis of diffraction data in terms of crystallite size.

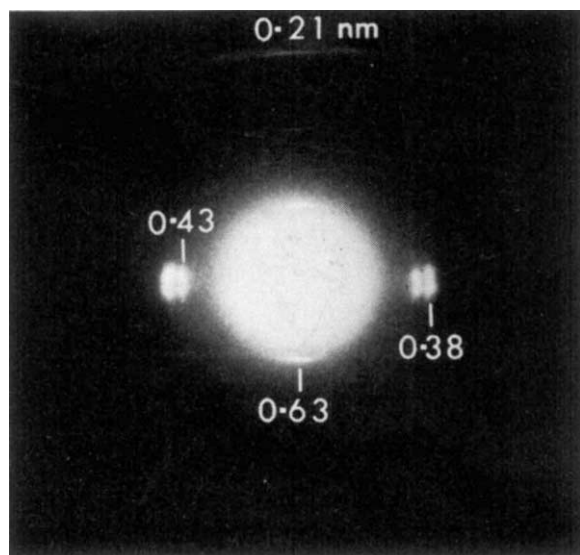


Fig. 1 Electron diffraction pattern from Kevlar 49.

Table 1 Crystallite size in Kevlar 49

Technique	Crystallite size (nm)
X-ray diffraction	7.9
Electron diffraction	8.1
Lattice fringes	8.6–22.5

Recently, E. I. du Pont de Nemours and Co. Inc. have developed the poly(*p*-phenylene terephthalamide) fibre Kevlar 49 composed of hydrogen-bonded sheets containing phenylene rings, whose mechanical properties approach those of PAN-based intermediate-modulus carbon fibres⁹. A preliminary X-ray diffraction survey indicates that the material is highly crystalline and there is no evidence for the small-angle meridional reflections which are traditionally associated with chain folding in polyamide and polyester

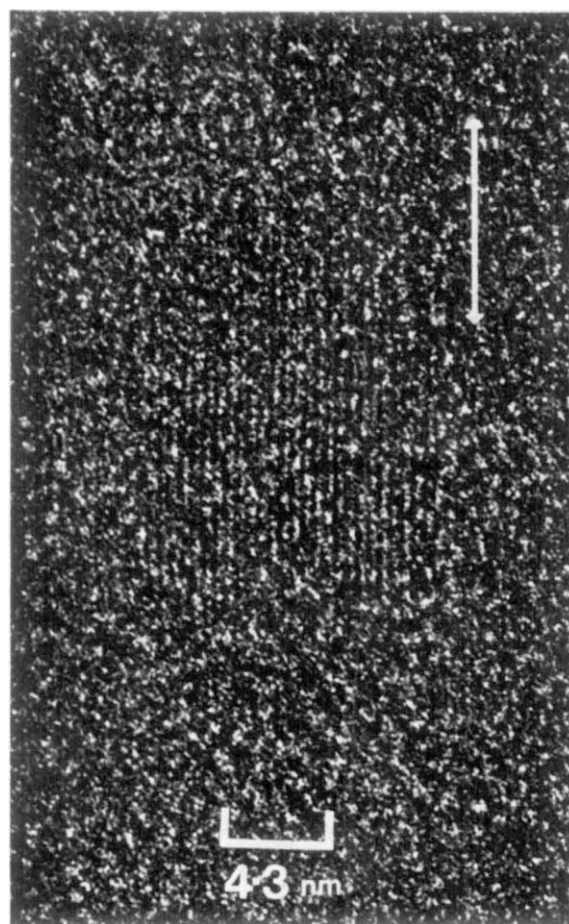


Fig. 2 Micrograph showing 0.433 nm lattice fringes in Kevlar 49 ($\times 3,200,000$).

fibres. Quantitative analysis of the equatorial X-ray diffraction trace by a method of profile resolution developed earlier¹⁰, indicates a crystallite width of 7.9 nm normal to the fibre axis, as obtained from the width of the resolved 0.433 nm reflection. The usual corrections were made to the overall intensity profile obtained by a step-scan diffraction procedure, and, after resolution, the peaks were corrected for instrumental broadening by a Stokes deconvolution method.

For electron-microscope examination in a Philips EM300, fibres of Kevlar 49 were fragmented by ultrasonic irradiation and the resulting material deposited on carbon-coated grids in the usual way. Electron diffraction patterns were

recorded which show a wealth of reflections. The principal diffraction maxima are shown in Fig. 1 where the innermost equatorial and three meridional reflections (even-order layer lines) correspond to Bragg spacings of 0.433, 0.388 nm, and 0.634, 0.317, 0.211 nm, respectively. These reflections decay at different rates during exposure to the electron beam; the 0.433 nm reflection is most stable but does not persist longer than about 120 s at a beam density of 3.28×10^{-4} A mm⁻². The equatorial intensity distribution was obtained from a corrected microdensitometer trace and analysed by a modified profile-resolution procedure developed for electron-diffraction analysis of selected areas in carbon fibres of heterogeneous structure¹¹. In spite of some degradation in the electron beam, the average crystallite size obtained from the breadth of the resolved 0.433 nm reflection is 8.1 nm, a value which compares favourably with the value obtained from X-ray diffraction analysis (Table 1).

Medium-resolution electron micrographs reveal breakdown of the fibres into extensive sheets which exhibit numerous kink bands, and, under dark-field conditions, a dense population of crystallites. Using carefully selected instrumental operating conditions developed from work on other beam-sensitive polymers⁷, high-resolution electron micrographs were recorded at magnifications calibrated by measurement of the 1.194 nm lattice fringes derived from the (201) planes in platinum phthalocyanine crystals. At the appropriate focal level, micrographs of Kevlar 49 clearly show lattice fringes related to the layer planes from which the 0.433 nm equatorial reflection originates; an example is illustrated in Fig 2. Although it must be emphasised that there is no exact one-to-one correspondence between layer planes and lattice fringes, an estimate of crystallite size in a direction at right angles to the fibre axis can be determined directly from such micrographs. Figure 2 shows an array of almost parallel lattice fringes approximately 12.5 nm in width, whereas in other micrographs more disordered lattices are apparent.

Measurement from a number of micrographs gives a size distribution within the range 8.6–22.5 nm (Table 1). It would appear that the averaged values obtained from fragments and fibres of Kevlar 49 are below the range measured directly. This comparison immediately questions the quantitative estimation of crystallite size by the classical diffraction methods, although it is possible that at present our lattice fringes are derived from the more perfect regions of the structure.

In conclusion we believe that the importance of this work is twofold. First, it provides a basis for the elucidation of relationships between molecular structure and physical properties in polymer systems, and second, it should enable us to test the validity of standard diffraction methods used to assess crystallite size and order.

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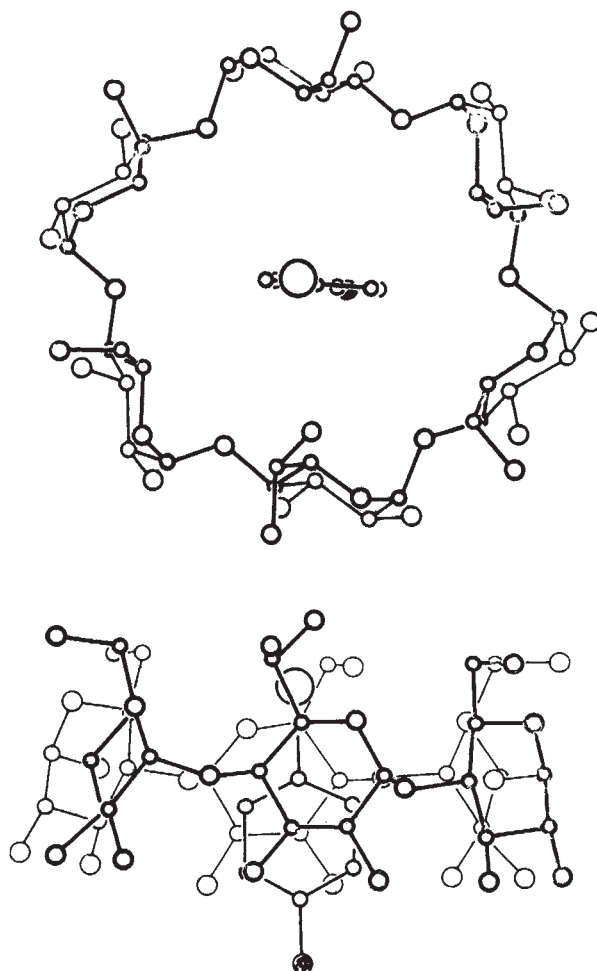
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Structure of α -cyclodextrin-*p*-iodoaniline complex

CYCLODEXTRINS, which are cyclic α -1,4-linked D-glucose oligomers, form a number of inclusion compounds with a variety of 'guest' molecules¹. The direct evidences for the formation of inclusion compounds have been derived from NMR spectra² and X-ray studies^{3–5}. α -Cyclodextrin is capable of forming a complex with a bulky molecule whose length is longer than the depth of the cavity of α -cyclodextrin. It is as yet not clear that which part of the molecule is included in and in what manner the molecule is bound to the interior of the α -cyclodextrin ring. We present here the structure of the α -cyclodextrin-*p*-iodoaniline(α -CDx-*p*-IAN) complex, (C₆H₁₀O₅)₈·C₆H₄NI·3H₂O.

The crystal is orthorhombic and the space group is P2₁2₁2₁, with unit cell dimensions; *a*, 13.659 (2) Å; *b*, 15.413 (4) Å; *c*, 24.436 (5) Å. The calculated density with *Z* = 4 was 1.610 g cm⁻³ and the observed density was 1.602 g cm⁻³ (flotation method). Intensities for 5,007 independent reflec-

Fig. 1 The structure of the α -cyclodextrin-*p*-iodoaniline complex. The circles, in order of decreasing size, represent iodine, oxygen and carbon atoms, and the black dots indicate nitrogen atoms.



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tions were measured on a Rigaku AFC four-circle diffractometer using graphite monochromatised MoK α radiation ($2\theta \leq 50^\circ$).

The structure was solved by the heavy atom method using 1,171 reflections with $2\theta \leq 30^\circ$. The three-dimensional electron density map calculated on the basis of coordinates of the iodine atom revealed the rough structure of the complex. The least-squares refinement of the atomic parameters which were estimated on the electron density map was unsuccessful. The accurate positions and directions of the six glucose units and the *p*-IAN molecule were determined by the block-diagonal rigid-body least-squares technique³. The O₆ atoms and water molecules were found on a difference Fourier synthesis. In successive refinements, one of the O₆ atoms was found to be disordered. Using 3,519 reflections with $|F_0| \leq 3\sigma(F)$, the final block-diagonal least-squares refinement with anisotropic temperature factors reduced the *R* value to 0.072. Fig 1 illustrates the structure of the complex.

Each glucose unit is in the C1 chair conformation, and all glucose units are α -1,4-linked; this result is in agreement with the structures of the other α -CDx complexes which were determined by X-ray analysis³⁻⁵. The *p*-IAN molecule is included in the cavity along the axis of α -CDx. The iodine atom and the benzene ring are situated in the cavity of α -CDx, while the nitrogen atom lies outside the cavity. The shortest distance between the benzene ring and α -CDx is 3.25 Å which is from an O₄ atom to a carbon atom of the benzene ring. This rather short intermolecular distance indicates that *p*-IAN is rigidly fixed in the cavity. The shortest distance from the iodine atom is 3.70 Å which is the distance to the disordered O₆ atom.

In the α -CDx-*p*-IAN complex, the six O₄ atoms of α -CDx lie very near the least-squares plane of themselves, and the maximum deviation from the plane is 0.13 Å. In the α -CDx-potassium acetate complex³ and the α -CDx-H₂O complex⁴, the maximum deviations are 0.01 Å and 0.98 Å, respectively. The average valence angle of α -1,4-linking oxygen atoms is 120° (2) which is in agreement with 119.1° in the α -CDx-potassium acetate complex and $119 \pm 3^\circ$ in the α -CDx-I₂ complex⁵. The distances between oxygen atoms O₂ and O₃ of adjacent glucose units are 2.68, 2.76, 3.00, 2.92, 2.98 and 2.87 Å which are the acceptable distances for hydrogen bonds. On the other hand, in the α -CDx-H₂O complex and the α -CDx-I₂ complex, there exist distances of 3.36 Å and 3.83 Å, respectively. In the α -CDx-*p*-IAN complex, all C₆-O₆ bonds are oriented to the outside of the cavity and the conformation is the same as that found in the α -CDx-I₂ complex; the conformational angles O₅-C₅-C₆-O₆ are $-75 \pm 8^\circ$ in the α -CDx-*p*-IAN complex and $-70 \pm 8^\circ$ in the α -CDx-I₂ complex.

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Received September 2, 1974.

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resemblance to a model species which is unpalatable (that is, distasteful or dangerous)¹⁻³. Predators learn to avoid the unpalatable model species after one or more adverse experiences with it, and any mimic species that resembles the model sufficiently closely will likewise be avoided.

The first examples of mimicry were documented in Brazilian butterflies¹—most additional examples and experimental work have been limited to mimicry in insects. Vertebrates exhibiting mimicry are rare, but include fish⁴, snakes^{5,6} and birds^{7,8}. A single example has previously been described in mammals: Shelford described a series of five squirrel species which mimic unpalatable treeshrew models in Borneo^{9,10}. The case described here is a possible additional example which, if confirmed, extends the phenomenon of mimicry to a large African mammal.

The genus *Hyaena* is represented in Africa today by two species: the striped hyaena, *H. hyaena*, inhabiting northern Africa; and the brown hyaena, *H. brunnea*, limited to southern Africa. Hyenas (the spotted hyaena, *Crocuta*, excluded) weigh 50–60 kg, and inhabit open dry plains and thorn scrub, live singly or in pairs, and are chiefly nocturnal. *Hyaena* has a sloping back, pointed ears, and an erectile mane. It has strong

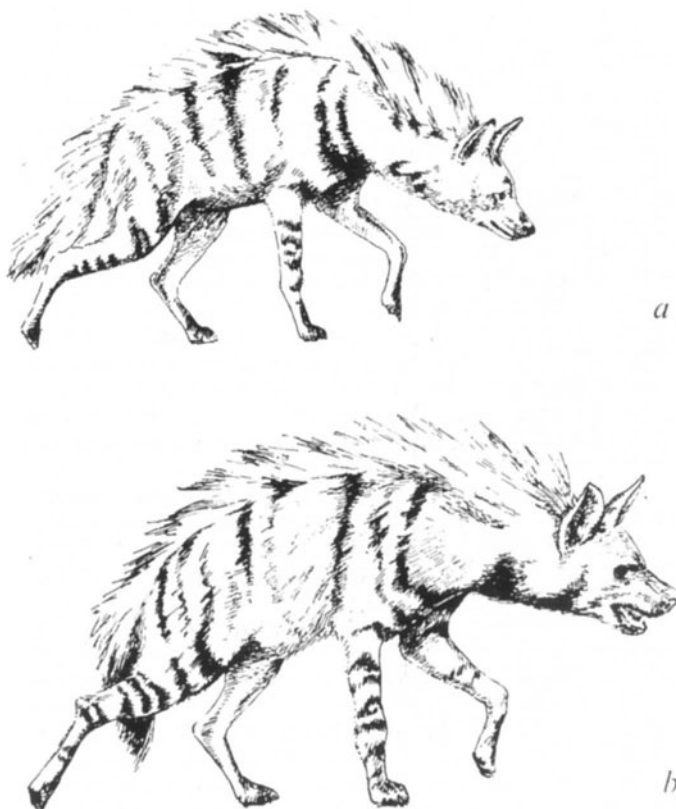


Fig. 1 External appearance of, a, the aardwolf, *Proteles cristatus*, and, b, the hyaena, *Hyaena hyaena*, both drawn to the same scale. Redrawn by P. Olsen from colour illustrations in Dorst and Dandelot²⁹.

teeth and jaws and it is known to kill live game occasionally, although it is primarily a scavenger.

The aardwolf *Proteles cristatus* is a much smaller, jackel-sized (12–15 kg) mammal with a discontinuous distribution: a northern population is found throughout most of East Africa, and a separate population inhabits southern Africa. *Proteles*, like *Hyaena*, has a sloping back, pointed ears, and a well developed erectile mane along the dorsal spine from neck to tail. This mane is composed of stiff hairs some 20 cm in length and, when erected, makes the aardwolf appear considerably larger than it actually is¹¹. *Proteles* inhabits open

Is the aardwolf a mimic of the hyaena?

MIMICRY is a phenomenon of evolutionary convergence or parallelism by which an edible mimic species gains some measure of protection from predators by virtue of its close

plains and thorn scrub, lives singly or in pairs, and is chiefly nocturnal. It has only vestigial cheek teeth and feeds largely on termites and other insects, and occasionally on carrion and rodents^{12,13}.

In his initial description of *Proteles*, Geoffroy St-Hilaire¹⁴ noted that its external appearance is very similar to that of *Hyaena*, a resemblance noted in virtually every subsequent description of the aardwolf (Fig. 1). Kruuk¹³ observed that *Proteles* and *Hyaena* look so similar that they are often confused. The resemblance of *Proteles* to *Hyaena* in body, mane and tail colour, and in the colour and development of stripes remains close in spite of considerable clinal variation in these characters; the pattern in each subspecies of *Proteles* resembling closely the sympatric subspecies of *Hyaena*¹⁴⁻¹⁶. Size is the only real difference in the external morphology of the two, and this is a notoriously difficult quantity to perceive in the field in the absence of some fixed comparative scale.

In addition, *Proteles* is found throughout its distribution in the same open plain or thorn scrub habitat as *Hyaena*, and its general behaviour is remarkably similar. The aardwolf and hyaena are both chiefly nocturnal, and live singly or in pairs. Even their kneeling defensive response to attack is similar¹¹.

The external similarity of *Proteles* to *Hyaena* extends also to most of the characters of internal anatomy that have been studied, indicating that *Proteles* is closely related to the hyaenas. The chromosomes¹⁷⁻¹⁹ and haemoglobin mobility²⁰ of *Proteles* are virtually identical to those of *Hyaena* and *Crocuta*, and the gyri and sulci of the cerebral hemispheres of the brain are arranged on exactly the same plan¹¹. The complete dental formula of *Proteles*, though not always fully developed, is the same as that of *Hyaena* and *Crocuta*²¹. The male reproductive tract of *Proteles* appears to be more similar to that of *Hyaena* than to that of *Crocuta*^{21,22}. *Proteles* differs from *Hyaena* principally in having a dentition much reduced in size, and in retaining the pollex (a digit lost in both *Hyaena* and *Crocuta*)²³.

The evolutionary lines leading to *Hyaena* and *Crocuta* appear, from the fossil record, to have been distinct since the Miocene²⁴. Fossils bearing on the evolution of the aardwolf have recently been discovered^{21,25} which indicate a Pliocene or earlier time of divergence of *Proteles* from the ancestral hyaenid stock, but the precise phylogenetic relationships among these three genera of Hyaenidae are not yet completely clear.

Two hypotheses of hyaenid relationships seem tenable. *Proteles* may have separated from the common ancestor of *Crocuta* and *Hyaena* before they separated (suggested by the shared loss of the pollex in the latter two genera), in which case the close external resemblance of *Proteles* to *Hyaena* is perhaps convergent. On the other hand, *Crocuta* may have separated from the *Proteles*-*Hyaena* stock before these two became differentiated (suggested by the reproductive tract and the close external resemblance), in which case the external resemblance of *Proteles* and *Hyaena* may reflect a parallel retention from a common ancestor having this appearance.

The nearly complete geographic sympatry, close external resemblance, and similar behaviour of *Proteles* and *Hyaena* are unusual in closely related forms, since speciation normally involves significant divergence in geographic distribution, in external appearance, or in behaviour. All of the conditions of Batesian mimicry are met by *Proteles*, and this may help to explain its distribution, appearance, and behaviour. Only the predator remains to be identified.

Leopards occur throughout the range of *Proteles*, they are most active at night, and they are predominantly visually oriented predators. Leopards routinely prey on jackals^{26,27} and, in view of their wide prey tolerance²⁸, might be expected to prey on *Proteles* as well. Because of its large size and strong skull, a *Hyaena* would be dangerous to an attacking leopard. The much smaller, weaker *Proteles* would be a poor match for a leopard, and it seems that an important component of the aardwolf's defence against predators may be its close external resemblance to the larger, more dangerous *Hyaena*. The ability of *Proteles* to erect its mane when excited would

further confuse a potential predator as to its actual size and identity.

This possible case of mimicry in a large mammal is unique, and deserves further investigation. Unfortunately, low population densities and nocturnal activity patterns make both *Proteles* and *Hyaena* difficult to study in the field. Additional study of their behaviour and ecology will be of great interest, and observation of any interactions with leopards of particular importance.

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Total human body protein synthesis in relation to protein requirements at various ages

THE intensity of body and tissue protein metabolism per kg declines with increased adult body size in mammals¹. This fall parallels a similar progressive decline in the intensity of energy metabolism²⁻⁴. It has also been concluded that protein metabolism per unit of body weight is about four to five times faster in young rats than in adult man¹; this pattern of change extends to cellular and subcellular aspects of protein metabolism, such as plasma albumin synthesis, liver RNA content and enzyme activity^{1,5}. Similarly, the rate of protein synthesis per kg total body weight declines during growth and development within a species, such as the rat⁶. This parameter again parallels the reduction in the intensity of energy metabolism which occurs during the growth period³.

Such surveys provide a general picture of protein metabolism in mammals and help to explain the metabolic basis for differences in dietary protein requirements both within and between the various species. The concepts involved should apply to man at various ages⁷⁻⁹. In previous studies with human subjects, however, different approaches have been used, some of which are no longer considered valid, and insufficient detail has been provided. It is therefore difficult to use the estimates to compare changes in body protein and energy metabolism with those for dietary protein needs throughout a man's life.

Using a common approach, we have tried to characterise the relationship which exists between protein and energy metabolism in man and to examine dynamic aspects of body protein metabolism with reference to dietary protein needs, during the period of rapid growth and development and the later years when senescence dominates body metabolism and function. The procedures, analytical methods and calculations of total body

protein synthesis have been described previously (refs 11, 12, and W.P.S., P.B.P., R. S. Goldsmith, N.S.S., and V.R.Y., unpublished). To quantitate the rate of total body protein synthesis we used a modification of the constant isotope method¹⁰, which is based on the continuous administration of ¹⁵N-labelled glycine and the analysis of urinary urea for ¹⁵N after a constant level of isotope enrichment has been achieved. The main difference in our experiments was that equal tracer levels of ¹⁵N-glycine were given orally at intervals of 3 h, rather than by continuous intravenous or intragastric infusion. We found that these two methods of infusion (continuous and at

Table 1 Total body protein synthesis rate in humans at various ages

Age group	No. of studies	Body weight (kg)	Age (range)	Total body protein synthesis (g kg ⁻¹ d ⁻¹)
Newborn (premature)	10	1.94±0.59	1-46 d	17.4±7.9
Infant*	4	9.0±0.5	10-20 months	6.9±1.1
Young adult	4	71±15	20-23 yr	3.0±0.2
Elderly	4	56±10	69-91 yr	1.9±0.2

*For comparative purposes, the data of Picou and Taylor-Roberts¹⁰ have been included.

intervals) give essentially identical estimates of total body protein synthesis. ¹⁵N-glycine (95 or 99 atoms per cent excess) was administered at a rate of 0.5 mg ¹⁵N per kg body weight per day, given during a 60-h tracer period in studies with young adults and the elderly.

Three age groups were studied. (1) Newborn, including six premature infants and one full-term baby. All were studied during a 30-36 h ¹⁵N tracer period within 1-45 days of life. One baby was studied at 1 and 13 days of age and another at 16, 31 and 45 days of age. (2) Healthy, normal young adults, three male and one female, 20-23 yr old. (3) Healthy, elderly women, 69-91 yr of age. Diets provided constant intakes of essential nutrients judged to be adequate for each age group. A more detailed description of the subjects, experimental details and diets will be published elsewhere. Urinary urea was isolated¹³ and analysed for ¹⁵N, following reaction with hypobromite¹⁴, with the aid of a dual collector, isotope ratio mass spectrometer (MS 11, Vacu-metrics, Waltham, Massachusetts).

Table 1 summarises results for body protein synthesis for the three age groups studied. The intensity of body protein synthesis declined rapidly during the first year of life, the rate in young adults being about one sixth that in premature infants. During later adult years protein synthesis (per unit of body weight) continued to decline but more gradually, the value for elderly subjects being 63% that for young adults. The differences among the older age groups reflect, in part, body compositional differences but this could not account for the marked decline in the synthesis rate between premature babies, full-term infants and young adults. Thus, as observed in experimental animals⁶ the intensity of protein metabolism in man declines throughout life.

To explore further the relationship between changes in the intensity of whole body protein synthesis and in energy metabolism we have recalculated the data on the basis of energy expenditure for each age group. For the prematures total energy intake was used for the calculation; for comparing adults, it was considered appropriate to use the basal metabolic rate. The results are summarised in Table 2. Differences observed in the intensity of protein metabolism among the various age groups (Table 1) are essentially eliminated when body protein synthesis is related to the energy expenditure for the different age groups (Table 2). These results confirm previous observations that protein metabolism is closely related to basal energy metabolism both within and between mammalian species^{1,4}.

Finally, the nutritional significance of these observations on whole body protein synthesis may also be assessed by comparing changes in dietary protein needs at various ages with decline in the intensity of whole body protein metabolism. The comparison

Table 2 Mean total body protein synthesis in relation to energy metabolism and Dietary protein allowances in premature babies, infants, young adults and elderly women

Age group	Dietary protein allowance* (g kg ⁻¹ d ⁻¹)	Protein synthesis (g) (per calorie†) (per g protein allowance)
Newborn (premature)	3.2	0.15±0.09‡
Infants§ (~1 yr)	1.3	5.4
Young Adult	0.57	0.11±0.01
Elderly	0.42	0.11±0.03

*Values for prematures are based on mean protein intake by the babies studied. Allowances for infants and young adults taken from FAO/WHO¹⁵ and for the elderly the allowance is based on the same method of estimation using unpublished data (W. D. Perera), on determined obligatory urinary and faecal nitrogen losses for these four subjects.

†Energy expenditure for prematures based on total energy intake. For young adults the values for basal metabolic rate were obtained by calculation from Altman and Dittmer¹⁶. In the elderly group basal metabolic rate was determined by indirect calorimetry.

‡Mean ± s.d.

§Values for protein synthesis taken from Picou and Taylor-Roberts¹⁰ for this age group.

provided in Table 2, using current estimates for daily protein needs¹⁵, shows a fall in the latter which parallels that for total body protein synthesis with progressive growth and during subsequent ageing. Hence, the amount of utilisable protein required to support body protein synthesis does not show a marked age-dependent change in man when needs are related to protein synthesis. Our data indicate that approximately 1 g of dietary protein is required to support the needs for 4 to 5 g of total body protein synthesis. This suggests that the efficiency of total dietary nitrogen utilisation is similar in the newborn, adult and elderly, and that the differences in protein needs, expressed per kg body weight, for the various age groups are related to differences in the amount of protein synthesised per unit time.

This conclusion may also apply equally to the individual dietary essential amino acids, except during the early neonatal period when histidine¹⁷ and cystine¹⁸ appear to be required as obligatory constituents of the diet because the enzymes responsible for endogenous synthesis, at rates commensurate with metabolic needs, have not fully matured.

These studies were supported by grants from the United States National Institute of Health. We thank the nursing staff of the MIT Clinical Research Center, Dr H. Zayas-Bazan and Miss Christine Bilmazes for their help and the subjects for their cooperation. (In addition to administrative approval of the Committees on the Use of Humans as Experimental Subjects of the Boston Hospital for Women and MIT, written informed consent was obtained from each baby's parents and the older subjects provided their written informed consent.) The help given by Dr William Cochran, Mrs Joyce Sylvester and the nursing staff of the Special Care Nursery at the Boston Hospital for Women during the studies with premature infants is appreciated.

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Mechanisms of cell fusion

We report new findings on the chemically-induced fusion of hen erythrocytes and discuss possible ways in which the lipid and protein/glycoprotein components of membranes may behave during the process of membrane fusion. Our observations are consistent with Poste and Allison's¹ idea that the aggregation of intrinsic membrane proteins is important in membrane fusion but we suggest that fusion may occur in regions of perturbed lipid bilayer which are thus denuded of intramembranous particles.

The intramembranous particles of unfixed T and B mouse lymphocytes have recently been reported to aggregate on incubation at 0° C in dimethyl sulphoxide (DMSO) or 25–50% glycerol²; glycerol-induced aggregation of membrane-intercalated particles has also been observed in *Entamoeba histolytica*³. To test Poste and Allison's hypothesis, we have therefore studied the effects of these agents on hen erythrocytes to determine whether they induce these cells to fuse. On incubation for 5 min at 37° C with 5 M DMSO (35%), the cells became spherical and binucleate cells were observed after 25–30 min. The number of fused cells and the number of nuclei per cell both increased on further incubation (Fig. 1). No fusion occurred on incubation for 45 min with 3 M (or lower concentrations of) DMSO. We anticipated that DMSO would cause cell fusion because it causes the formation of large membrane blisters devoid of intramembranous particles in lymphocytes². The plasma membranes of fibroblasts treated *in vitro* with oleylamine also blister away from the underlying cytoplasmic organelles, and their plasma membranes usually fuse in these regions⁴.

Stewart and Turner⁵ have previously reported, although without comment, an apparent fusion between pairs of human erythrocytes observed in electron micrographs of cells treated with 40% glycerol. In our experiments multinucleated cells were observed on incubation of hen erythrocytes for 22 and 5 h at 37° C in modified Eagle's basal salt medium at pH 7.4 (ref. 6), containing Dextran 60 C (80 mg ml⁻¹) and glycerol (1.5 or 2.5 M, respectively). Dextran 60 C facilitated fusion induced by these quantities of glycerol but in control incubations without glycerol the dextran was not in itself fusogenic⁶. In the absence of dextran most of the glycerol-treated erythrocytes became swollen and were then lysed: only a few cells fused. Fusion induced by DMSO and glycerol, like that induced by fusogenic lipids⁶ was calcium-dependent (1.8 mM Ca²⁺) and was inhibited by EDTA (5 mM).

Since glycerol causes cell fusion, other polyols have been

investigated for fusogenic properties. Sorbitol fuses hen erythrocytes, numerous multinucleated cells being present after 2 h incubation in 2.5 or 3 M sorbitol dissolved in the standard buffer (free from Dextran 60 C). Higher and lower concentrations of sorbitol were less effective. Multinucleated cells have also been observed with mannitol (1.25 M, 6 h incubation), ethylene glycol (20%, 5 h incubation) and sucrose (1 M, 5 h incubation). The non-fusogenic Dextran 60 C (molecular weight approximately 90,000) greatly facilitated fusion by sucrose. In contrast, long incubation (30–48 h) of the cells

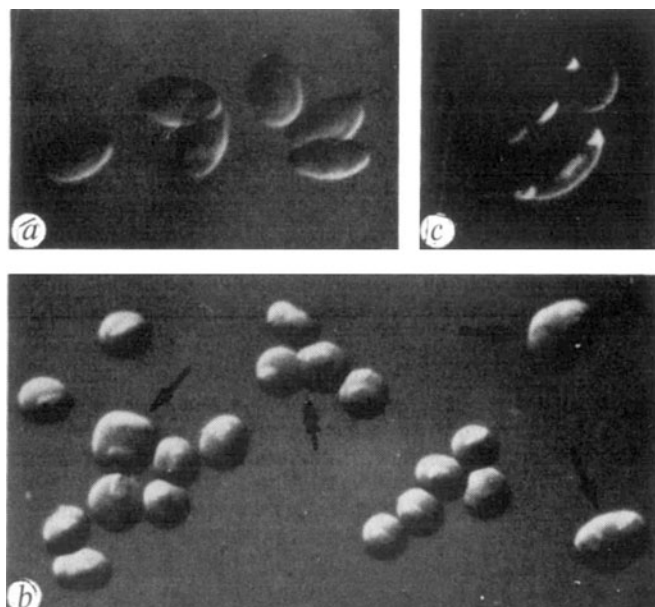


Fig. 1 Light micrographs ($\times 672$) of hen erythrocytes: *a*, cells (approximately 3.5×10^8 cells ml⁻¹) incubated at 37° C at pH 7.4 in Eagle's basal salt solution buffered with sodium cacodylate⁶; *b*, cells incubated in the buffer for 30 min at 37° C with 5 M dimethyl sulphoxide (DMSO), showing the cell adhesion which precedes cell fusion (double arrow) and three binucleate cells (single arrows); *c*, a multinucleated cell after incubation for 90 min as in *b*. Nomarski differential-interference-contrast microscopy.

with 80–100 mg ml⁻¹ of a high molecular weight dextran (2×10^6) was itself capable of yielding many multinucleated cells. Polyethylene glycol (molecular weight 6,000), which has recently been shown to fuse plant protoplasts⁷, is also capable of causing extensive cell fusion with hen erythrocytes.

Aggregation of membrane proteins may occur either by disorganising membrane lipids with a micellising agent such as lysolecithin⁸, or by increasing the fluidity of membrane lipids by heating⁹, or by the insertion of a low melting fatty acid or ester⁶. Whether glycerol induces protein clustering by interacting with the lipids or the proteins of membranes is problematic. The addition of glycerol to a lipid film at an air-water interface will, however, lower the surface potential of a condensed or an expanded film and also further expand a partially-expanded film¹⁰.

Poste and Allison¹ indicate that complete fusion of membranes, as observed in cell fusion, may be regarded as an extension of partial fusion observed in intercellular junctions. Particle aggregation in junctions presumably involves stable intermembranous protein-protein interactions as well as intramembranous protein clustering. In contrast, only the latter may be significant in membrane fusion, and protein clustering may be followed by lipid-lipid interactions between membranes, leading to a loss of membrane individuality and allowing membrane fusion to proceed. This interpretation is consistent with the fact that sonicated protein-free phospholipid vesicles can fuse readily under specified conditions^{11–13}.

Whether or not endogenous enzymes (for example, lipase¹⁴) are involved in one or more stages of chemically- or virus-

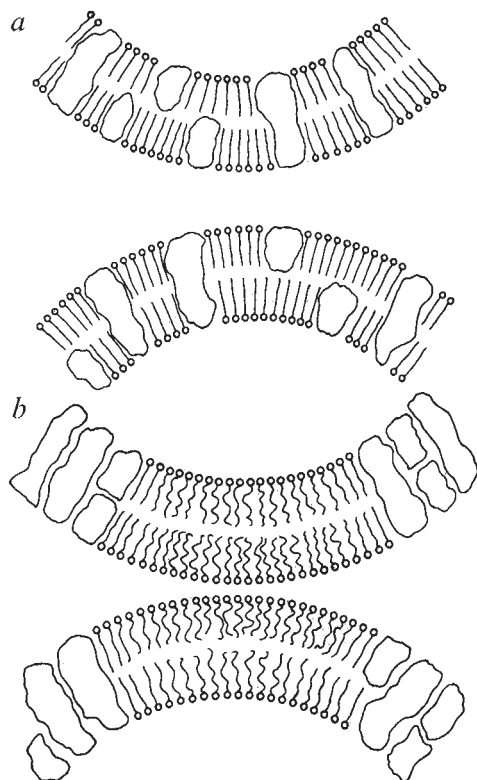


Fig. 2 Possible relationships between the lipid molecules and the intrinsic proteins of biological membranes during membrane fusion: *a*, two membranes in close proximity which do not fuse because the proteins of the membranes are randomly arranged in their respective lipid bilayers; *b*, membranes in which fusion may now proceed, by the interaction and intermingling of their lipid molecules, after the emergence of protein-free areas of lipid bilayer as a result of aggregation of the intrinsic proteins in both membranes. Compared with *a*, the lipid molecules of the membranes that are about to fuse have been perturbed; the lipids of *b*, are either more fluid (as illustrated), or, in the extreme case, are rearranged in micellar form (not shown).

induced cell fusion remains debatable. We suggest therefore that cell fusion induced by exogenous chemical agents seems to involve, *inter alia*, the following events. First, a perturbation of the bilayer structure of membrane lipids may occur which increases the fluidity of the lipid region, or in the extreme case results in micelle formation. These changes in the lipid structure of the plasma membrane then allow the aggregation of intramembranous protein/glycoprotein particles. Second, the interaction and intermixing of the disturbed lipid molecules of closely apposed membranes in regions denuded of intramembranous proteins and glycoproteins may allow adjacent cells to fuse (Fig. 2). Concanavalin A, polylysine and protamine, which have been reported to induce aggregation of membrane proteins, cause erythrocytes to aggregate but not to fuse. In some instances, however, the induction of protein clustering may be sufficient to lead to fusion (such as that of *Drosophila* cells induced by concanavalin A, ref. 15), if aggregation of the proteins gave rise to perturbed, rather than stable, pools of lipid bilayer.

Comparable mechanisms to that suggested here may apply to fusion involving the membranes of subcellular organelles. For example, during mucocyst secretion by exocytosis in *Tetrahymena*, fusion occurs between a region of plasma membrane, sequestered within a rosette of particles, and a smooth area of mucocyst membrane free from particles¹⁶. The complex sequence of changes observed in mucocyst secretion may therefore be a specialised means of allowing the lipid bilayers of closely apposed membranes to interact in regions denuded of intramembranous proteins.

This work was supported by a Research Grant from the Medical Research Council. We thank Mr R. A. Parker for preparing Fig. 2.

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Received July 16; revised November 27, 1974.

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Infection of differentiating muscle cells with influenza and Newcastle disease viruses

REPLICATION of influenza viruses is believed to require functions provided by the host cell nucleus because first, influenza replication is inhibited by actinomycin D and α amanitin, which prevent transcription of complementary RNA from virion RNA in the first few hours following infection^{1–4}; and second, migration of viral proteins between host cell cytoplasm and nucleus occurs during the virus replicative cycle^{5–8}.

We therefore examined the replication of influenza viruses in skeletal muscle cells, as the latter undergo a characteristic differentiation resulting in the formation of syncytial myotubes, the nuclear DNA synthesis of which has terminated^{9,10}, and the RNA metabolism of which has changed considerably^{11–14}. Such myotubes have been reported to be resistant to direct infection by the DNA viruses polyoma and SV40 (ref. 15) and the RNA Rous sarcoma virus¹⁶, all of which interact with host nuclear DNA during their replication.

Breast muscle from 12-d chick embryos was treated with trypsin and cells were cultured in 60 mm Petri dishes as previously described¹⁷. The appearance of uninfected cell cultures during the course of differentiation is illustrated in Fig. 1. One day after plating, cultures contained predominantly dividing single cells, myoblasts (Fig. 1a), and low frequency myotube formation was also observed. This was followed one day later by a period of rapid cell fusion, which had ceased by about 40 h after plating. If carefully selected batches of horse serum were employed, about 70 to 80% of the myoblasts fused (Fig. 1b). After plating for 3 d the myotubes broadened and areas between myotubes filled with a population of single cells which had failed to fuse (Fig. 1c). The myotubes later became striated, and started contracting spontaneously (not shown).

Influenza virus strain A/AA/60(H2N2) was studied, using Newcastle disease virus (NDV) as control, as NDV is not believed to require any host nuclear genetic information for its replication (reviewed in ref. 18). Both viruses were passaged in chicken eggs, and virus seeds received were similarly propagated initially in the allantoic cavity of eggs. Subsequently, the viruses were grown in monolayers of primary chick kidney

cells¹⁹ to obtain tissue culture-grown virus stocks which were used to infect muscle cell cultures.

For infection of muscle cell cultures the growth medium was removed, cells were washed twice with Hanks' balanced salt solution (HBSS) and 1 ml of inoculum added. After 30 min adsorption at room temperature and removal of residual inoculum by washing twice with HBSS, growth medium containing 5% horse serum added. Cultures were incubated at 40° C, and examined for cytopathic effect and also for their ability to adsorb chicken red blood cells (haemadsorption).

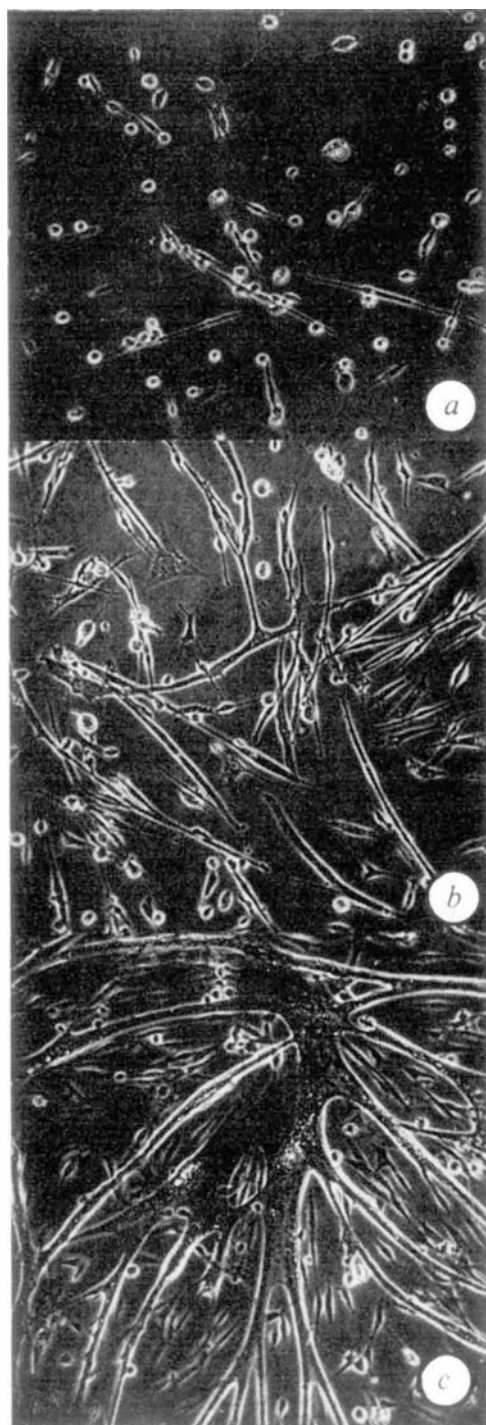


Fig. 1 Kinetics of myotube formation in cultured chicken skeletal muscle cells. *a*, Single cells present in prefusion culture 25 h after plating. *b*, Syncytial myotubes and residual single cells in post-fusion cultures 40 h after plating. *c*, Enlarged myotubes and residual single cells 60 h after plating. Small refractile cells in each case are residual chicken red blood cells after these uninfected cultures were tested for haemadsorption.

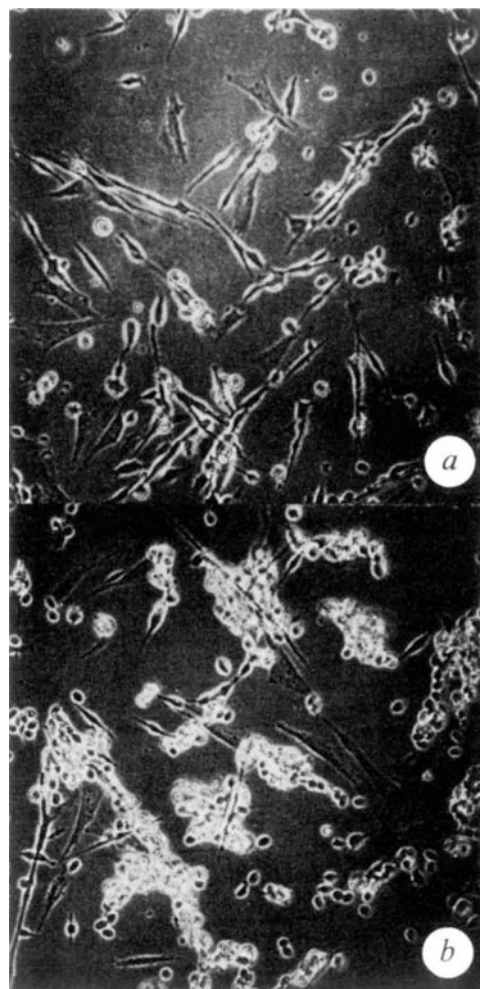


Fig. 2 Lack of influenza replication in prefusion muscle cell cultures. Cultures of skeletal muscle cells were infected 17 h after plating with influenza virus A/AA/6/60(H2N2) or NDV (La Sota) at a multiplicity of 5 to 10 EID₅₀ per cell. After infection for 9 h, cultures were flooded with a suspension of chicken red blood cells, and 10 min later unadsorbed red blood cells removed by gentle washing. *a*, HAD⁻ appearance of culture infected with influenza virus. *b*, Culture infected with NDV having many HAD⁺ single cells and early myotubes. Several identified fields were examined before and after haemadsorption, thereby confirming that many of the HAD⁺ single cells were of myoblast type. Similar results to those shown in the figure were obtained using multiplicities of infection ten times greater.

Figure 2 shows cultures infected with influenza and NDV when observed for haemadsorption 9 h after infection. With the former, very few, if any, single cells were haemadsorption-positive (HAD⁺) in contrast to the NDV cultures where a large proportion of single cells and early myotubes were very strongly HAD⁺. Very similar qualitative findings were obtained in cultures 24 h after infection (not shown).

Table 1 shows this difference in ability of the two viruses studied to replicate in prefusion muscle cells. At 9 h after infection with influenza virus 2% of the cells could be scored as HAD⁺, compared with 1.4% for the control cultures; 24 h after infection with influenza virus 0.8% of the cells could be scored as HAD⁺, compared with 0.26% for the control cultures. It is difficult to interpret this slight difference between values for control and infected prefusion cell cultures as a result of virus replication, in view of the uncertainty with which clusters as small as three red blood cells may be unequivocally identified, and the possibility that small amounts of residual virus from the initial inoculum might cause low levels of haemadsorption. From the results in Table 1 we conclude that any replication of the influenza virus in myoblasts was at best marginal.

Table 1 Efficiency of infection of prefusion muscle cell culture by influenza and Newcastle disease viruses (NDV)

Virus	Time after infection (h)	Total cells	HAD ⁺ cells	% Cells HAD ⁺	% Cells HAD ⁺ , difference from control
None (= control)	9	440	6	1.4	—
None (= control)	24	390	1	0.26	—
Influenza virus A/AA/6/60	9	450	9	2	0.6
Influenza virus A/AA/6/60	24	640	5	0.78	0.52
NDV (La Sota)	9	380	190	50	48.6
NDV (La Sota)	24	420	110	26	25.7

Cells infected with 5 to 10 EID₅₀ of virus were incubated for the time shown before examination for haemadsorption. Each result shown is the total from three or four fields, examined in two separate experiments. Cells were scored as HAD⁺ if they were associated with clusters of three or more red blood cells. Control cells were included to provide a baseline, as small clusters of red blood cells were observed at low frequency in control cultures.

In contrast, NDV exhibited a very significant degree of replication in myoblasts; 9 h after infection 50% of the total cells were HAD⁺ ($P \leq 0.001$, compared with control or influenza-infected cultures). It should be emphasised that for calculations in Table 1 each myotube was scored as a single cell, although the syncytial cell had been formed by the fusion of some 10 to 20 myoblasts. Accordingly the efficiency of infection calculated for NDV is probably an underestimate, being biased by the fact that the HAD⁺ early myotubes in the cultures were scored

as single cells. Reduction in the proportion of HAD⁺ cells in cultures infected with NDV by 24 h after infection (Table 1) may therefore be an artefact resulting from the increasing degree of cell fusion that occurs in the cultures with time. Even without taking this factor into account, the results in Table 1 show that the efficiency of replication by NDV in myoblast cultures is at least 50 to 80 times greater than for the influenza virus. This has been confirmed by dose-response experiments (M.C.O'N., and A.P.K., unpublished).

We also examined replication by the myxoviruses in post-fusion muscle cell cultures, and for this purpose cultures were maintained without infection until myotube formation had reached its maximum. Infection with influenza virus and NDV was then performed in an identical manner to that employed with prefusion cell cultures. Figure 3a illustrates the high frequency with which myotubes supported influenza virus replication. When cultures were examined about 9 h after infection; virtually every myotube was HAD⁺, whereas of the single cells still present in the culture less than 5% were HAD⁺. Many myotubes and single cells were also HAD⁺ in post-fusion cultures infected with NDV (Fig. 3b).

Similar post-fusion cell cultures were maintained for 24 h after infection with influenza virus or NDV. In this case, influenza virus infected cultures were almost totally devoid of myotubes, which detached from the cell layer, and were observed floating in the culture medium. Since it had been shown that myotubes were infected with influenza (Fig. 3a) it was concluded that prolonged incubation of myotubes after influenza infection resulted in a cytopathic effect and probably cell death. This was supported by the observation that, in post-fusion cultures examined 24 h after influenza virus infection, a few remaining myotubes were strongly HAD⁺ and were beginning to detach from the surface of the culture dish (Fig. 4a). The almost total lack of myotubes 24 h after influenza virus infection is clear from comparisons either with uninfected control cultures of similar age (for example, Fig. 4c); or with similar cultures infected with the lentogenic NDV, which did not cause myotube death by 24 h after infection (Fig. 4b).

These observations establish for the first time that, unlike those viruses which require cellular DNA synthesis for their replication, the RNA viruses of influenza and Newcastle disease can directly infect myotubes of cultured chicken skeletal muscle, in which the cells have terminated DNA synthesis^{9,10}. In addition, under the conditions described, it was demonstrated that, before syncytia formation, cultured muscle cells are inefficiently infected by influenza virus, based on the inability to detect synthesis of the viral haemagglutinin.

Lack of influenza replication in the prefusion muscle cells is unlikely to be an artefact of the multiplicity of infection or host-adaptation compared with NDV. Experimental conditions were identical for pre- and post-fusion cell cultures, the single exception being the state of cellular differentiation. Multiplicities of infection were similar for the influenza and Newcastle disease viruses, and the observations reported were highly reproducible using several different batches of tissue

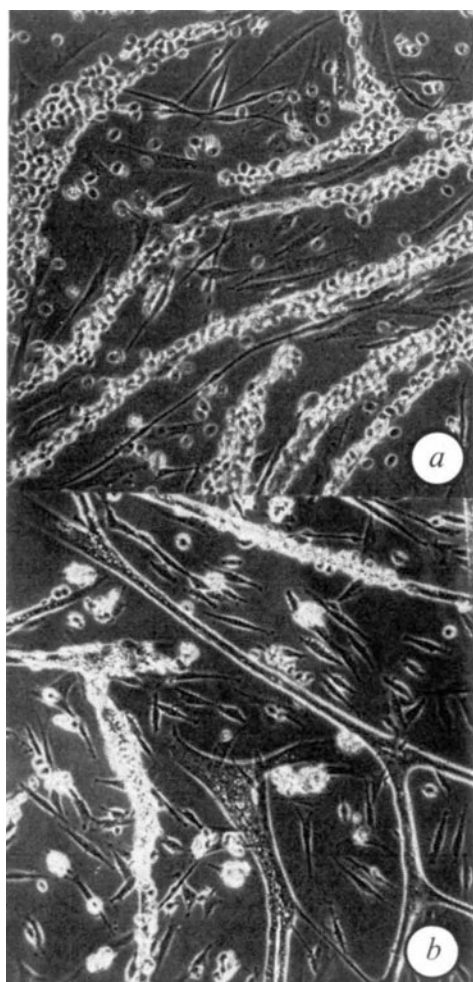


Fig. 3 Detection of virus growth in post-fusion muscle cell cultures. Conditions were similar to Fig. 2, except that cell cultures were not infected until 40 h after plating, by which time maximum formation of myotubes had occurred. *a*, Culture infected with influenza virus and containing myotubes which were almost all HAD⁺, whereas residual single cells were HAD⁻. *b*, Cultures infected with NDV and containing HAD⁺ single cells and myotubes.

culture-grown virus, although it is not yet known whether the same behaviour holds true for other strains of influenza.

The observation that the state of cellular differentiation can

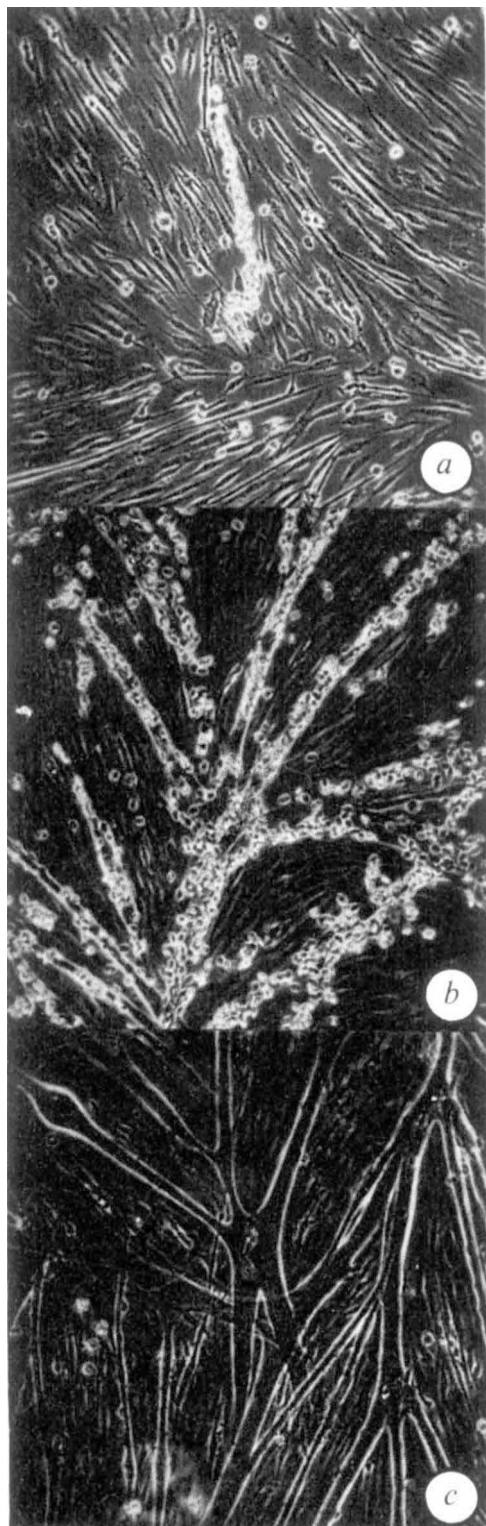


Fig. 4 Cytopathic effect of influenza virus on myotubes in cultured muscle cells. Conditions were similar to Fig. 3a and b, except that infected cultures were maintained for 24 h before testing for haemadsorption. *a*, Cultures infected with influenza virus, virtually devoid of all myotubes, but which contains an occasional HAD⁺ myotube, showing early cytopathic effect and also beginning to detach from the cell sheet. Single cells are HAD⁻. *b*, Culture infected with NDV, and containing HAD⁺ myotubes and single cells. *c*, Control culture.

affect the ability of muscle cells to support replication of an influenza virus is novel. Since orthomyxoviruses and paramyxoviruses both initiate infection by attachment to sialic acid-containing receptors on cell membranes followed by fusion of virus and cell membranes²⁰⁻²², it seems improbable that the low efficiency with which the influenza virus infected myoblasts was the result of a cell surface restriction. The different behaviour of NDVs and influenza viruses suggests rather that the restriction on influenza virus replication observed in myoblasts was related to functions in the host cell nucleus. That this restriction ceases after myotube formation might well be one result of the changes that occur in the nuclear activities of the muscle cells at the time of myoblast fusion. Further experimentation is required to justify this hypothesis; if valid, the system we have described should prove valuable in studying the role of the host cell nucleus during influenza replication, and the nature of changes in the muscle cell nucleus during differentiation.

We thank Janet Myers for the technical assistance. Seed of influenza virus was obtained from Dr H. F. Maassab of the University of Michigan School of Public Health, and the lentogenic Newcastle disease virus strain La Sota from Dr M. Bratt of the University of Harvard Medical School.

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The reversal of methotrexate toxicity by thymidine with maintenance of antitumour effects

METHOTREXATE (MTX) is effective in the treatment of acute lymphoblastic leukaemia¹, choriocarcinoma², Burkitt's lymphoma³, and a number of other solid tumours⁴⁻⁶. An increased therapeutic index for methotrexate with delayed citrovorum factor (CF) has been reported in experimental tumour systems⁷ and in patients with head and neck cancer⁸ and osteogenic sarcoma⁹. The biological basis for this increased therapeutic index is not known, but delay in administration of citrovorum factor is a crucial feature. We have studied the effects of thymidine (TdR) on the toxicity and therapeutic effect of MTX in mice bearing leukaemia L1210. CF was included in the experiments as a positive control, and allopurinol because this drug has been reported to impair the antitumour effects of MTX⁹.

L1210 Leukaemic cells (10^5) were injected intraperitoneally into BDF₁ male mice, and MTX, CF, TdR, or allopurinol injected intraperitoneally at least 24 h after the inoculation of leukaemic cells. The animals were weighed

Table 1 The effect of thymidine and citrovorum factor in methotrexate toxicity in normal mice

	Drug	Dose (mg kg ⁻¹)	Schedule	No. of deaths/No. of mice	Day of death
Expt 1	MTX	200	qd × 1	1/8	8
		250	qd × 1	7/8	7 ⁷
		300	qd × 1	6/8	5,6 ⁵
	TdR	500	tid × 4	0/8	—
	MTX	200	qd × 1	1/8	6
	TdR	500	tid × 4		
	MTX	250	qd × 1	1/8	6
	TdR	500	tid × 4		
	MTX	250	qd × 1	5/8	6 ⁴ ,7
	TdR	500	tid × 4		
Expt 2	MTX	300	qd × 1	1/8	7
		400	qd × 1	4/8	7 ³ ,8
	TdR	500	tid × 3	0/8	—
	MTX	300	qd × 1	0/8	—
	TdR	500	tid × 3		
	MTX	400	qd × 1	0/8	—
	TdR	500	tid × 3		
	MTX	400	qd × 1	0/8	—
	CF	500	bid × 3		
	MTX	400	qd × 1		

The first dose of CF or TdR in combination experiments was given at the same time as MTX.

qd × 1 = once.

tid × 3(4) = 3 times per day for 3(4) days.

Superscript numbers, number of animals dying.

weekly and observed for toxicity and survival. Since animals dying of drug toxicity died before the leukaemic control animals, this allowed the distinction between toxic and tumour deaths to be made⁷.

Table 1 shows that TdR (500 mg kg⁻¹ intraperitoneally three times per day for 4 d), the first dose being given at the same time as MTX, substantially reduced MTX toxicity in normal animals. Figure 1 shows that TdR similarly prevented MXT toxicity in tumour-bearing animals but did not inhibit the antitumour effects of MTX; if allopurinol was added to the MTX-TdR combination, the life of tumour-bearing animals was not prolonged. If the first TdR administration was delayed for 12 h after MTX treatment, the selective rescue effects of TdR were still observed,

and the therapeutic effect was superior to that produced by delayed CF administration. TdR pellets implanted subcutaneously also protected normal tissues from MTX toxicity although the antitumour effects were not inhibited (ref. 10 and M.H.N.T., B.L.B., and E.F., unpublished).

These experiments indicate that in mice TdR prevents MTX toxicity in normal host tissues but does not inhibit its antitumour effects. MTX-TdR seems to be superior to MTX-CF in the treatment of animals with L1210 leukaemia. The biological basis for these observations has not been established. The data suggest that MTX treatment causes thymidylate starvation in normal tissues, which can be reversed by the administration of excess TdR; and since the tumour is not rescued by thymidine, it is possible that

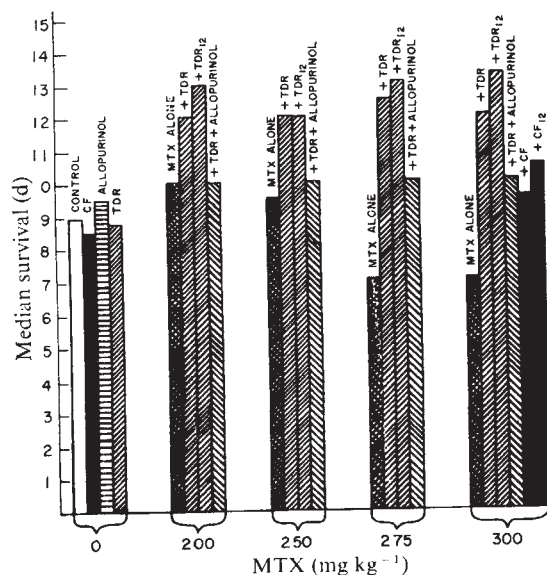


Fig. 1 The effects of MTX, TdR, CF and allopurinol in L1210 leukaemia. Histogram of median survival of control and drug-treated animals. The numbers of animals in each group was at least six, and the first administration of drug was 24 h after intraperitoneal transplantation of 10⁵ ascites cells. MTX was given as a single injection; TdR (500 mg kg⁻¹ three times per day for 3 days) was given at the same time as MTX, alone, with allopurinol, or the first dose was delayed for 12 h after MTX (TdR₁₂). Citrovorum factor (200 mg kg⁻¹) was given at the same time as MTX or the first dose was delayed for 12 h (CF₁₂). MTX caused no significant increase in survival time. MTX-TdR and allopurinol was significantly less effective than MTX-TdR alone: at MTX 200, 250, 275 mg kg⁻¹, $P < 0.001$; at MTX 300 mg kg⁻¹, $P < 0.05$ (Mann-Whitney tests). Methotrexate sodium (4-amino-N¹⁰ methyl pteroylglutamic acid sodium), and citrovorum factor (5-formyl tetrahydrofolic acid, calcium leucovorin) were obtained from Lederle Labs, Pearl River, New York. Allopurinol (4-hydroxypyrazolo (3,4-d) pyrimidine) was obtained from Burroughs Wellcome, London and TdR from Nutritional Biochemical Corp.

MTX causes additional disturbances in tumour tissues. Hryniuk¹¹ has reported that MTX causes death in rapidly proliferating L5178Y cells in culture as a result of a lack of purine. Grindey and Moran⁹ observed that allopurinol, which elevates plasma and tissue purine levels, reduced the antitumour effects of MTX in mice with L1210 leukaemia. All these data indicate that MTX treatment may cause purine starvation in L1210 tumour cells *in vivo*. The retention of non-dihydrofolate reductase protein by MTX-Sepharose indicates that MTX may have multiple sites of action¹². It is known, however, that reduced folates are 1 carbon donors in thymidylate and *de novo* purine biosynthesis. As bone marrow cells utilise predominantly pre-formed purines synthesised by the liver¹³, for nucleic acid synthesis it seems possible that TdR may rescue normal proliferating host tissues, which do not rely on *de novo* purine biosynthesis, but not rescue tumour cells in which *de novo* biosynthesis may be the major source of purines.

This work was supported in part by a National Cancer Institute Grant. M.H.N.T. has an MRC Travelling Fellowship.

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Microsomal enzyme induction by methadone and its implications on tolerance to methadone lethality

MANY investigators have sought to explain narcotic tolerance as a result of increased metabolic inactivation of the drug. No evidence for disposition tolerance has yet been reported, however¹⁻⁸. On the contrary, tolerance to most narcotic effects seems to be adaptive or cellular tolerance, that is a decrease in pharmacological effects even when enough drug is in contact with target cells in the brain to produce marked effects in naive animals⁷. We wish to report, however, that metabolic inactivation of methadone, a widely used synthetic narcotic, does increase, with a resulting tolerance to its toxic properties.

Subcutaneous or oral administration of methadone has been shown to prevent one sign of narcotic withdrawal in

mice tolerant to and dependent on morphine after implantation of a morphine pellet for 3 d (ref. 8 and L. M., unpublished). After 6 d of oral methadone treatment (100 mg kg⁻¹ of the hydrochloride salt), these mice display tolerance to the lethal effects of methadone, but not to those of morphine (Table 1). This observation suggests the existence of disposition tolerance to methadone, as an increased LD₅₀ for morphine, as well as methadone would have been expected had cellular tolerance developed in these animals.

The liver seems to be the chief site of metabolism for methadone⁹, which is a substrate for the rat and rabbit hepatic microsomal N-demethylase enzymes¹⁰. We therefore examined the activity of N-demethylase in the livers of mice maintained on the same regimen as that described above. The activity of N-demethylase was assayed in the hepatic 12,000g supernatant fraction¹¹ 6 d after removal of the morphine pellet. Ten micromoles of D,L-methadone were used as substrate. The 6-d oral administration of methadone brought about a nearly twofold increase in the activity of the enzyme. This increase in enzyme activity may account for the elevation of the methadone LD₅₀ which was determined in identically treated animals. Since N-demethylation plays only a minor role in the inactivation of morphine⁷, the fact that the LD₅₀ for morphine was unchanged is not surprising.

To demonstrate that the elevation in the methadone LD₅₀ was the result of increased N-demethylase activity following methadone administration and not residual cellular tolerance brought about by the morphine pellet treatment, we gave naive mice phenobarbital intraperitoneally (50 mg kg⁻¹ d⁻¹), which increases the activity of a variety of microsomal enzymes¹². After 3 d of treatment, a nearly sixfold increase in N-demethylase activity occurred (Table 2). There was a concomitant fourfold increase in the oral LD₅₀ for methadone. A less dramatic but significant increase of 55% in the intraperitoneal methadone LD₅₀ was also noted. In contrast, the intraperitoneal morphine LD₅₀ was not significantly affected by the barbiturate treatment. Since these mice had never been exposed to narcotics, it seems that a change in disposition of methadone did occur.

The increased activity of N-demethylase observed as a result of methadone treatment is not a consequence of the morphine pellet treatment since morphine treatment has been shown to lower N-demethylase activity significantly². To demonstrate the ability of methadone to increase N-demethylase activity, naive mice were given oral doses of methadone (50 mg kg⁻¹) daily. After 24 h, a 50% increase in the activity of N-demethylase was noted. By day 6, the activity was increased nearly twofold (compare 0.770 ± 0.124 with 1.419 ± 0.124 μmol of formaldehyde per 30 min per liver).

Table 3 demonstrates the similarities between the effects of methadone and phenobarbital on liver protein content and liver weights. Both treatments caused a significant elevation in the liver weights and in the protein of the 12,000g supernatant fraction and of the microsomal fraction of the liver.

Table 1 Effects of repeated methadone treatment on methadone and morphine lethality and activity of liver N-demethylase in mice rendered tolerant by implantation of a morphine pellet

Treatment	Methadone LD ₅₀ ± s.e. (mg kg ⁻¹ , p.o.)	Morphine LD ₅₀ ± s.e. (mg kg ⁻¹ , i.p.)	N-demethylase activity (μmol formaldehyde/ 30 min/liver ± s.e.)
Water	120 ± 4 (24)	404 ± 27 (24)	0.770 ± 0.124 (11)
Methadone	198 ± 21* (24)	407 ± 34 (24)	1.42 ± 0.12* (11)

Mice were implanted with 75 mg morphine base pellets for 3 d (ref 8). Treated animals were given 100 mg kg⁻¹ of D, L-methadone HCl in water (5 mg ml⁻¹) orally once a day for 6 d after removal of the pellet. Controls were given equivalent volume of water. All determinations were made 24 h after the last dose. LD₅₀s were calculated according to the method of Miller and Tainter²⁰. N-demethylase activity was assayed using approximately 5 mg of microsomal protein per assay from the 12,000g supernatant fraction of mouse livers. The assay procedure of Fouts¹¹, using 10 μmol (2 mM) of D, L-methadone HCl as substrate, was used. Numbers in parentheses indicate number of animals in each group.

* Significantly different from controls, *P* < 0.05.

i.p., Intraperitoneally; p.o., orally.

Table 2 Effect of phenobarbital treatment on methadone and morphine lethality and activity of liver N-demethylase in naive mice

Treatment	Methadone LD ₅₀ ± s.e. (mg kg ⁻¹)	Morphine LD ₅₀ ± s.e. (mg kg ⁻¹)	N-demethylase activity (μmol formaldehyde/30 min/liver ± s.e.)
Saline	84.0 ± 4.6 (24), p.o. 40.0 ± 2.8 (24), i.p.	480 ± 44 (24)	0.598 ± 0.026 (5)
Phenobarbital	304 ± 42* (24), p.o. 62.0 ± 2.6* (24), i.p.	547 ± 23 (24)	3.58 ± 0.23* (5)

Mice were injected intraperitoneally with 50 mg kg⁻¹ phenobarbital in saline (5 mg ml⁻¹) once a day for 3 d. Controls were injected with saline. All determinations were made 24 h after the last injection using the same procedures as in Table 1. Numbers in parentheses indicate number of animals in each group.

* Significantly different from controls, $P < 0.05$.

The increases in microsomal protein and N-demethylase activity after methadone seem to be typical of the phenobarbital type of induction of microsomal enzymes¹².

The principal metabolites of methadone are 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (M₁) and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (M₂)^{13,14}. Both are formed by N-demethylation of methadone and non-enzymatic cyclisation and, in the case of M₂, further N-demethylation¹⁵. These metabolites have been isolated from rat bile^{14,16} and urine¹⁶, as well as from human plasma^{17,18}, bile^{14,17} and urine^{13-15, 17, 19}. They have no demonstrated analgesic activity¹⁴ and, therefore, probably play no role in the prevention of the narcotic abstinence syndrome. Thus, the increase in the activity of N-demethylase following repeated administration of methadone should increase the rate of

drug (50 mg kg⁻¹). Subcutaneous administration of methadone brought about a much smaller decrease in this half-life.

These observations may have clinical implications for methadone maintenance in which doses are often greater than one-half the minimal lethal dose in non-tolerant subjects. Rapid inhibition of microsomal metabolism can follow after exposure to a number of drugs and environmental agents^{26,27}. Morphine and heroin, for example, are abused by subjects on methadone maintenance and are effective microsomal enzyme depletors^{2,28}. Furthermore, the occurrence of microsomal enzyme inhibition is compounded when methadone is taken orally, a route which is associated with a slow onset and prolonged duration of drug action. Thus, toxic sequelae may not develop until the subject has

Table 3 Effects of phenobarbital and methadone on liver weights and protein in liver fractions

Treatment	Liver weight ± s.e. (g)	Protein in 12,000g supernatant fraction of liver ± s.e. (mg)	Protein in microsomal fraction of liver ± s.e. (mg)
Naive mice			
Saline (5)	1.42 ± 0.06	159 ± 7	62.6 ± 2.8
Phenobarbital (5)	1.68 ± 0.08*	203 ± 11*	85.9 ± 4.3*
Mice implanted with morphine pellet			
Water (10)	1.37 ± 0.06	109 ± 8	50.4 ± 3.6
Methadone (14)	1.69 ± 0.06*	127 ± 6*	60.4 ± 2.7*

Naive mice were injected with phenobarbital or saline according to the procedure in Table 2. Mice implanted with 75 mg morphine base pellets were given either D,L-methadone HCl or water orally for 6 d according to the procedure in Table 1. All determinations were made 24 h after the last dose. The microsomal fraction was obtained by centrifuging 1-ml aliquots of the 12,000g supernatant fraction at 105,000g for 1 h. Proteins were determined by the method of Lowry *et al.*³⁰. No significant differences were noted in the body weight data. Numbers in parentheses indicate number of animals in each group.

* Significantly different from controls, $P < 0.05$.

inactivation of the narcotic and contribute to the development of tolerance to some methadone effects.

There is circumstantial evidence for the role of metabolism in the termination of the pharmacological actions of methadone. Basic metabolites of methadone are significantly elevated in the faeces of rats after repeated methadone administration²⁰. These compounds are probably M₁ and M₂ since both have a higher pK_a than methadone²¹ and represent the greatest portion of methadone metabolites^{14,15}. More recently, pretreatment with methadone for 15 d was shown to increase the biliary excretion of M₁ by 50% and advance its peak excretion time¹⁶. Since biliary excretion accounts for the majority of these compounds in the faeces²², these two observations support the possibility that increased activity of microsomal enzymes is the consequence of the repeated administration of methadone.

Alvares and Kappas²³ showed that phenobarbital pretreatment of rats results in an enhancement of demethylation of methadone *in vitro* by liver microsomal preparations and that the analgesic effect of methadone decreases concomitantly. No increased N-demethylase activity was demonstrated following chronic administration of methadone (20 mg kg⁻¹, intraperitoneally), however. Peters²⁴ was also unable to demonstrate enhanced demethylation after daily intraperitoneal injections of methadone (5 mg kg⁻¹) in rats. In contrast, Misra *et al.*²⁵, using an oral rather than a parenteral route, observed that the biological half-life of methadone in the plasma and brain of rats was reduced more than fourfold after repeated administration of the

left the methadone maintenance centre for the day.

This work was supported in part by a US Public Health Service Training Grant and a grant from the US National Institute of Mental Health.

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Received March 19, revised November 26, 1974.

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Increased neonatal mortality in offspring of male rats treated with methadone or morphine before mating

METHADONE (($\pm$)-4, 4-diphenyl-6-dimethylamino-3-heptanone hydrochloride) is a potent (addictive) analgesic with pharmacological effects which are qualitatively similar to those of morphine, although it is far more effective than morphine when given orally. Its ability to diminish the severity of the abstinence syndrome resulting from heroin withdrawal led to the introduction of methadone in the chemotherapy of narcotic addiction¹⁻³. Little is known, however, about its long term toxicity effects. We report that treatment of male rats with either ($\pm$)-methadone HCl (METH) or morphine sulphate (MS), given orally, for 24 h before mating to untreated females increases the neonatal (21-d) mortality of their offspring.

Sixty naive female Charles River (CD) albino rats (which had not been previously treated with the drug) were mated to 26 male rats which had received 13, 18 or 38 mg kg⁻¹ d⁻¹ METH during

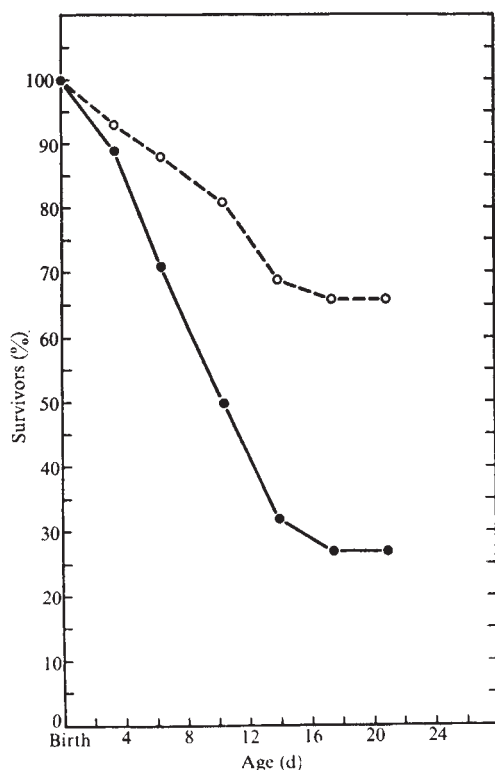


Fig. 1 Neonatal (21-d) survival of 166 live offspring sired by male rats pretreated with MS (○) and 145 live offspring sired by male rats pretreated with METH (●). Twenty-six per cent of the offspring sired by METH pretreated males and 66% of the offspring sired by the MS pretreated males survived. Of the live offspring of untreated males 90-95% survived for 21 d.

the previous 24 h. Another 60 naive female rats were mated to 26 other male rats similarly treated with 22, 47 or 55 mg kg⁻¹ d⁻¹ MS. Females were exposed to treated males for 7 consecutive days.

Figure 1 shows the marked difference in the 21-day mortality of the offspring. Those from MS-treated males (six fostered and ten unfostered litters) had 57 deaths out of 166 live births (34% mortality), whereas those from METH-treated males (seven fostered and seven unfostered litters) had 107 deaths out of 145 live births (74% mortality). This difference is very significant ($\chi^2 = 46.77$, $P < 0.001$). The METH offspring ($n = 145$) died at the rate of 8 per day from days 4 to 14, whereas the MS offspring ($n = 166$) died at a rate as high as 6 per day only between days 11 and 14, suggesting that a different lethal mechanism is operating. Overall mortality in both groups is considerably greater than the 5.9% rate in 1,645 offspring resulting from matings of untreated males and females, and reared by untreated foster mothers^{4,5}.

We also investigated the effects of pretreating male rats for 24 h with METH given 0-24 h or 25-48 h before mating with naive females ($n = 12$ in each group). Males were first placed in a cage and provided with a 5% sucrose drinking water

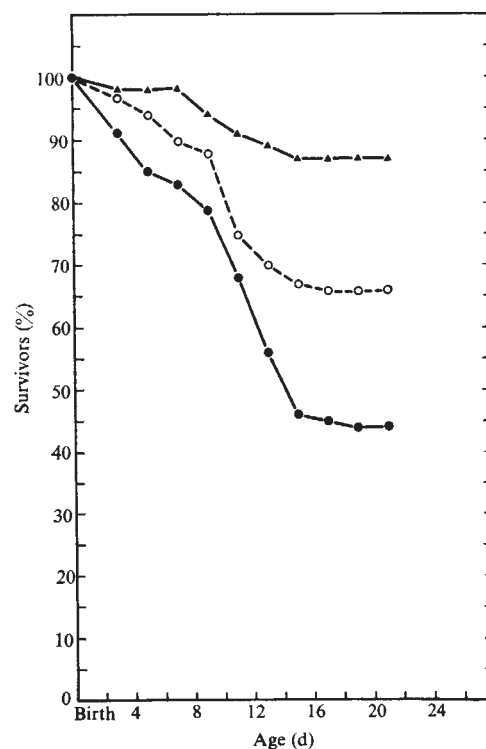


Fig. 2 The 21-d survival of the offspring of naive females mated with male rats pretreated with either no drug (▲) or with low-dose METH (○, 25 mg kg⁻¹ d⁻¹) or high-dose METH (●, 52 mg kg⁻¹ d⁻¹) during the 24-h period before mating. These three mortality rates differ significantly ($P < 0.01$) from each other.

solution containing 0.67 mg ml⁻¹ or 0.067 mg ml⁻¹ METH. The 'high-dose' METH males received 52 mg kg⁻¹ d⁻¹, the 'low-dose' METH males 25 mg kg⁻¹ d⁻¹. A third group received sucrose solution alone. Each male remained in this cage with four females for 24 h. (The offspring of these females are not part of the present study.) Males were then moved to a second cage provided with drinking water with neither drug nor sucrose, containing four drug-naive females, the offspring of which are identified as the '0-24 h' offspring, as the males had received METH in that period prior to siring these offspring. After 24 h with these females each male was then moved to a third cage provided with pure drinking water and containing

four more naive females, the offspring of which are identified as the '25-48 h' offspring. After 24 h with these females each male was then returned to the initial cage containing the drinking water with 5% sucrose solution (with or without METH) and the cycle repeated. Each naive female was exposed to males for 6 consecutive days.

The neonatal (21-day) mortality of the offspring of the three groups of males is shown in Fig. 2. In 21 days, 13% of 132 offspring sired by control males died, whereas 56% of 100 offspring sired by high-dose METH males died (difference very significant: $\chi^2 = 47.08$, $P < 0.001$) and 34% of 100 progeny of low-dose METH males died (difference from controls significant: $\chi^2 = 13.59$, $P < 0.01$, and from high-dose of METH progeny: $\chi^2 = 8.91$, $P < 0.01$). The neonatal mortality of 244 offspring of males which had received METH 25-48 h before mating was only 25%. This did not differ significantly ($\chi^2 = 3.41$, $P > 0.05$) from the 21-day mortality of their controls (17%). Of the ten litters sired by control males only one had a death rate in excess of 30% (Fig. 3), whereas of the eight litters sired by the high-dose METH males, six litters lost 40% or more of the offspring born alive. The low-dose methadone litters had a smaller death rate than the high-dose METH offspring. These data demonstrate that the increased mortality of the METH-sired offspring is not simply a consequence of the deaths of the offspring of a small proportion of the males, but of an increased mortality in virtually all litters.

Pretreatment of male rats with METH before mating thus produces a significant increase in neonatal (21-day) mortality of the offspring of the METH-treated males and naive females when compared to control matings. Our data show a dose-response relationship for this phenomenon as well as evidence

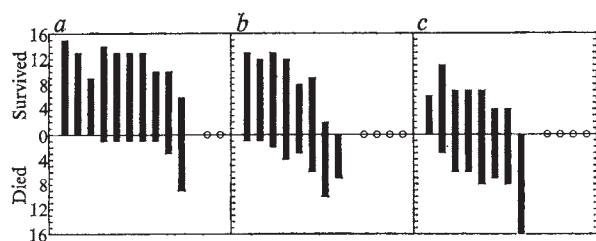


Fig. 3 Survival and death in each litter of offspring born to females mated to (a) controls and males receiving (b) low- and (c) high-dose METH 0-24 h before mating. The vertical black bars show the survival or death of the live offspring from each litter. ○, Females which did not deliver offspring.

that the lethal effect is seen primarily in offspring sired during the first 24 h following administration of METH to the male. Morphine produced a similar but less marked effect. An urgent problem in medicine is the fact that 1-3% of all infants born have one or more unexpected congenital anomalies which cannot be explained by classical teratology. Our preliminary results suggest that it is necessary to investigate not only the drugs which were administered to the mother before and during pregnancy, but also those drugs used by the father. This approach may contain the answers to some of the unexplained problems of childhood growth and development.

We thank Norman F. Sourdoff for technical assistance and Sue Mauldin Smith for statistical computations.

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Received July 1; revised December 3, 1974.

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Physiology of visual cells in mouse superior colliculus and correlation with somatosensory and auditory input

THE two main targets of the mammalian optic nerve fibres are the lateral geniculate body and the superior colliculus (optic tectum). From studies with various techniques, and in several mammalian species including the cat¹⁻⁵, monkey⁶⁻⁹, rabbit¹⁰⁻¹², rat¹³, and ground squirrel¹⁴ three major functions of the superior colliculus have been described. In the superficial layers the visual input is processed in a specific way; in deep layers several sense modalities, chiefly visual, auditory and somatosensory, are brought together; stimulation of the tectum results in an orienting of the animal's eyes, head or body towards a location corresponding topographically to the part of the tectum stimulated.

Gordon¹ has shown that for a given location in the cat tectum there is a good correlation between the positions of visual receptive fields and the directions from which maximal auditory responses are evoked. She also found a correlation between preferred directions of stimulus movement in the two modalities. The agreement between visual and somatosensory cells was much looser, although visual receptive fields near the vertical midline were correlated with tactile fields on the face, and temporal visual fields, with tactile fields on the body or legs. She rarely observed responses from whiskers. Cats move their eyes and especially their heads extensively, however, and a very close relationship between retinotopic and somatosensory maps was perhaps not to be expected.

We have studied the superior colliculus in the mouse, a small compact animal with very little eye movement and relatively little head movement; a mouse rather turns its whole body towards an interesting object. The major part of the mouse's visual field is crossed by an elaborately developed somatosensory organ, the vibrissae. Whisker movement in the mouse is very rapid, but the excursions are small, so that the whiskers bear a relatively constant relationship to the visual field. One should therefore not be surprised to find a close topographical relationship between the tectal projections from retina and whiskers.

In our study, ten mice of the C57BL/6J strain were used. Throughout the experiment a mouse was kept under light anaesthesia (pentobarbital and chlorprothixene) and was neither paralysed nor artificially respired. (For exact procedures, see Dräger¹⁵.) The head was held fixed by means of a small metal block glued to the skull, thus leaving the ears free. Visual receptive fields were mapped on a translucent tangent screen placed at a distance of 10.5 cm from the mouse. Electrolytically polished tungsten electrodes were used for recording. Electrode tracks, most of which were perpendicular to the tectal surface, were marked by several small electrolytic lesions (2 μ A for 2 s) and reconstructed histologically. A total of 323 recordings were made in 48 penetrations. Of these records, 145 were probably from tectal cells. The remaining 178 recordings, from unit clusters or poorly resolved units, were useful for purposes such as topographical mapping of the tectal surface.

Cells in the superficial layers had small visual receptive fields (average diameter 9°) whose locations in the visual field varied according to a topographical map similar to that described for other vertebrates¹⁶: the nasal visual field projected anteriorly and the upper visual field medially. These cells responded only to visual stimulation, with best responses to a slowly moving small spot of any shape. One-quarter of the cells preferred one direction of movement; for most cells the preferred direction was upward. Deeper in the tectum most visual cells had very different receptive-field properties, with large fields (20°-60°), sluggish and transient responses to small moving objects, little or no response to large stimuli, and often a directional selectivity with preference for upward movement. Characteristically these deeper-layer cells tended

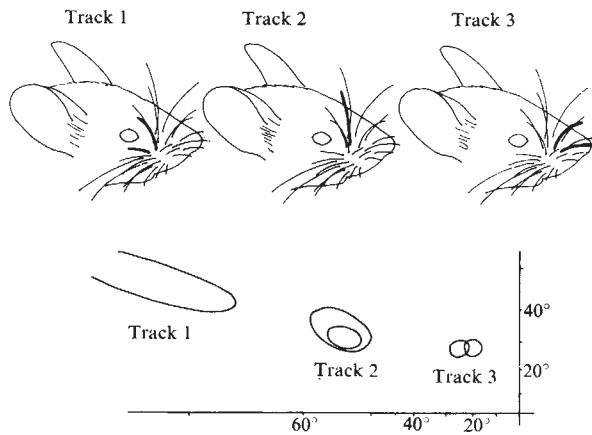


Fig. 1 Correlation between somatosensory and visual receptive fields recorded in three successive electrode penetrations. Lower part of diagram shows visual receptive fields recorded in superficial tectal layers and mapped on tangent screen. Mouse was facing the tangent screen at right angles, 10.5 cm behind it. Origin of coordinate axes marks intersection with the long axis of the mouse. Upper part of the diagram indicates whiskers giving strongest responses in the same electrode tracks at a deeper level; these whiskers are drawn as thicker lines.

to habituate if the same stimulus was repeated, but fired again if the spot or object was moved through a different part of the field. Similar visual receptive field properties in the superior colliculus have been described in other species^{5,6,10,12,13}.

At a slightly deeper level, in the stratum griseum mediale, cells responding to somatosensory or auditory stimulation were first encountered intermixed with visual cells, and with cells responding to two (or rarely to all three) modalities. Still deeper in the tectum, over a distance of several hundred μ m, somatosensory or auditory modalities tended to take over completely until, in some penetrations, the electrode again entered an area of mixed responses. There was no segregation of somatosensory and auditory cells into sub-layers; cells responding to a given modality seemed rather to be arranged in clusters with some intermixing at the boundaries between clusters.

Somatosensory responses were obtained about twice as frequently as auditory. In most penetrations the tactile receptive fields were located on one or a few whiskers. Gently tapping one whisker evoked a short burst of spikes with little difference in response as the direction of hair deflection was varied.

The most striking feature was a very consistent relationship in any one electrode track between the location of visual receptive fields and that of somatosensory receptive fields. A series of three electrode tracks in one experiment is illustrated in Fig. 1. The axes in this illustration represent the horizon and the vertical midline of the mouse, drawn on the tangent screen so as to cross at the extended longitudinal axis of the mouse. Numbers on the axes mark degrees of eccentricity. Visual receptive fields in the three tracks are outlined on the screen. In the upper part of the figure, the whiskers giving the strongest responses from cells deeper in the tectum are drawn more heavily than the others. In the first track there was a clear correlation between temporal visual fields and posterior whiskers; moving nasally in the visual field in tracks 2 and 3 was accompanied by movement to more anterior whiskers. In other series of penetrations a similar correlation was seen between visual receptive-field positions and the whiskers that evoked the best responses at deeper levels in the tectum. If the visual receptive fields were progressively lower in a set of tracks, the whisker fields likewise moved down. By drawing the projections of the whiskers through the eye on to the tangent screen, we could show that for a given perpendicular penetration the visual receptive fields were crossed by just the whiskers that evoked maximal responses from the somatosensory cells

deeper in the tectum. This correlation held not only for neighbouring visual and somatosensory cells, but also for single cells driven by both modalities.

In parts of the tectum in which visual receptive fields were far peripheral, and no whiskers lay in the way, somatosensory responses at deep levels were evoked from other parts of the body. Visual fields far down, where the mouse might see its own paw, were associated with tactile fields on the dorsum of the paw. Far temporal visual fields were correlated with somatosensory fields on the flank, shoulder and ear; here an upward movement in the visual field was paralleled by upward movement in the somatosensory map, from the flank to the tip of the ear.

Reactions to auditory stimulation were found less frequently than to tactile stimuli and they tended to be less well localised. Auditory responses could be evoked by complex sounds rich in high frequencies like clicks or crackling noises. Usually only sounds generated from a direction contralateral to the colliculus were effective in driving auditory cells. In eight out of thirteen cells the auditory receptive field in the horizontal plane was restricted to an angle of about 50°–150°, which included the visual and somatosensory receptive field of cells recorded simultaneously or in the close vicinity.

The mouse superior colliculus thus contains three topographical maps, superimposed and roughly in register, of the surroundings as observed through visual, somatosensory and auditory modalities. We assume that this system serves to orient the animal to an interesting stimulus whatever the sense modality.

We thank David Freeman for designing the electronic equipment and Claire Wang for histological assistance. The work was supported by a grant from the National Institutes of Health and by grants from the Rowland Foundation, Inc., and the Esther A. and Joseph Klingenstein Fund, Inc.

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Conductance of channels opened by acetylcholine-like drugs in muscle end-plate

Most recent views of the way in which acetylcholine (ACh) can cause ion channels to open postulate that the channel can exist only in one or other of two distinct conformations, open or shut^{1–4}. In their simplest form, these theories predict that a channel, once it is open, will have the same conductance whichever drug caused it to open. We have estimated average single channel conductances for four cholinomimetic agonists, and find this prediction is not confirmed.

The mean single-channel conductance, and mean lifetime of the open state, have been estimated at the voltage-clamped end-plate of the frog (summer *Rana pipiens*) cutaneous pectoris muscle. Methods similar to those of Anderson and Stevens⁵, were used with the addition of Normarski interference optics,

which make the end-plate clearly visible. The drugs shown in Fig. 1 were applied iontophoretically to the end-plate, and samples of the end-plate current fluctuations taken at $1,024\text{ s}^{-1}$. The mean single-channel conductance, γ , was estimated from the total variance, s_I^2 , of the current fluctuations, as $\gamma = s_I^2/m_I(V - V_{eq})$, where m_I = mean end-plate current induced by the drug, V = membrane potential and V_{eq} = equilibrium potential. This method assumes that the fluctuations represent the random opening and closing of channels, and that no appreciable contribution to s_I^2 occurs outside the frequency range examined in our experiments (1–500 Hz).

Measurements in both normal and in glycerol-pretreated muscle fibres showed that V_{eq} was the same (near 0 mV), within a few mV, for all the drugs in Fig. 1. Measurements on normal fibres were obtained by depolarising in small steps of a few mV, spaced by about 10 s, as the contraction threshold (about –55 mV) was approached, until about –30 mV was reached at which point the excitation-contraction mechanism was inactivated. The variance was estimated from the variance of the digitised noise record, and as

$$s_I^2 = \int_{-\infty}^{\infty} S(f)df = \int_0^{\infty} G(f)df$$

where $S(f)$ is the two-sided, and $G(f) = 2S(f)$ the one-sided spectral density computed from the fluctuations. These methods gave very similar results.

The results are shown in Table 1. The means for all experiments are given, because the results were reproducible from day to day. For example, three separate experiments with suberylcholine (SubCh) gave $\gamma = 28.4 \pm 1.6$, 28.2 ± 2.2 , and 29.2 ± 2.4 pmho.

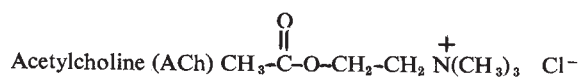
It is clear from these results that the mean single-channel conductances, γ , are not the same for all drugs, but that they vary over an approximately twofold range for the drugs tested. In all seven experiments in which SubCh and ACh were tested on the same cell, it was found that SubCh had a larger γ than ACh, the mean ratio being 1.19 ± 0.04 . SubCh also had a longer mean open lifetime than ACh, by a factor of 1.7 (Table 1), the same as the factor found by Katz and Miledi⁷. The agonists 3-phenylpropyltrimethylammonium (PPTMA) and 3-(*m*-hydroxyphenyl)propyltrimethyl ammonium (HPTMA), which are full agonists as judged by their ability to cause contraction of the frog rectus abdominis muscle⁸, have a substantially smaller γ than either ACh or SubCh.

Katz and Miledi⁷ have already shown that the mean length of time for which the ion channel stays open may differ over an approximately tenfold range for different drugs, at the frog muscle end-plate. Contrary to the predictions of the simple two-state theories, it seems that differences in agonist action that can produce a tenfold difference in the mean time that a channel remains open, can also influence the mean conductance of the open channel. For the drugs tested at least, γ varies much less than the mean open-channel lifetime, τ , from drug to drug. It is also intriguing that a large single channel conductance seems to accompany a large open lifetime, but not enough drugs have been tested yet to allow any generalisation to be made.

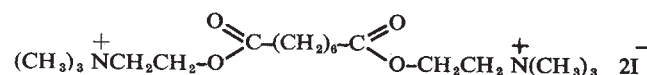
Table 1 Values of mean single-channel conductance (γ) computed from integrated spectral densities for current fluctuations, and values of mean open-channel lifetime, τ , at 10–15°C and a membrane potential of between –60 and –80 mV, computed as $1/(2\pi f_c)$ where f_c = half-power frequency.

	γ (pmho)	τ (ms)
SubCh	28.6 ± 1.0 (22)	5.6 ± 0.3 (8)
ACh	25.0 ± 0.9 (8)	3.2 ± 0.3 (6)
HPTMA	18.8 ± 0.8 (19)	1.0 ± 0.06 (13)
PPTMA	12.8 ± 1.1 (7)	0.83 ± 0.02 (3)

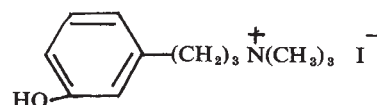
The frog Ringer solution contained tetrodotoxin (100 nM). All results are on normal (not glycerol-treated) fibres. The table gives the mean of values on all cells $\pm$ s.e., with the number of determinations in parentheses.



Suberylcholine (SubCh)



3-(*m*-hydroxyphenyl) propyltrimethyl ammonium (HPTMA)



3-phenylpropyltrimethyl ammonium (PPTMA)

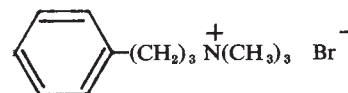


Fig. 1 Drugs applied iontophoretically to *Rana* muscle end-plate.

The spectra of current fluctuations produced by ACh and SubCh could be fitted well with a single time constant ' $1/f^2$ ' model^{1,5}. The mean single channel conductance, estimated from the zero frequency asymptote of spectral density⁵, as $\gamma = S(0)/2m_I(V - V_{eq})\tau$, agreed well with estimates of γ from the noise variance in the case of these drugs. The spectra of the other two agonists, however, had a component at low frequency, in addition to the major high frequency component. The sum of two single time-constant spectra was found to fit these results. The 'mean open lifetimes' in Table 1 were derived from the high frequency component, but whether or not they estimate accurately the lifetime of an actual species depends on the explanation for the two components that is adopted. It is possible that several species of channel exist with different mean open times, corresponding, conceivably, with the binding of different numbers of agonist molecules. But it can be shown quite simply that if the channels all have the same conductance when they are open, then the value of γ calculated from the noise variance should be this conductance, regardless of how the channels are distributed among the species of different mean open lifetimes⁶.

Two classes of explanation can be imagined for the observation that mean single channel conductances are not the same for all drugs. It could be that the channel can exist in several distinct open conformations, each of which has a different conductance, and that different drugs favour different conformations. On the other hand, it could be that the open channel has essentially the same conformation for all drugs, but individual drugs have, for example, somewhat different effects on local field strength or local pH so that each drug affects the pK of ionisable groups, or access of ions to the channel, in a slightly different way. It has been reported⁹ that reduction of the ACh receptor with dithiothreitol changes ACh voltage fluctuations in a way which suggests that the primary change is a lowered single-channel conductance.

Our conclusions depend, of course, on the correct estimation of γ . It is conceivable, for example, that current fluctuations actually reflect receptor occupancy by the drug⁵, and that the channel oscillates more rapidly than we can observe between open and shut states while it is occupied. In this case our estimates of γ would be only a lower limit of the true open channel conductance.

We thank Dr R. B. Barlow and Dr A. Ungar who gave us the drugs. This work was supported by the United States Public Health Service and the Wellcome Trust.

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Received September 23; revised November 2, 1974.

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Reversible spectral change of squid retinochrome by salts

THE cephalopod retina contains two kinds of photosensitive pigments, rhodopsin and retinochrome. Retinochrome was first found in the inner segment of squid visual cells¹. More recently it was reported that the outer segment might also contain retinochrome together with rhodopsin². The chromophore of retinochrome, all-*trans* retinal, is predominantly converted into the 11-*cis* form by illumination. It has been suggested that retinochrome supplies 11-*cis* retinal for the regeneration of cephalopod rhodopsin in the dark, by acting as an isomerase¹. The validity of the mechanism, however has not yet been established *in vitro* or *in situ*.

The chemical behaviour of retinochrome has been studied and compared with that of rhodopsin^{1,3}. Retinochrome has an absorption spectrum that depends on pH, and is sensitive to hydroxylamine and NaBH₄, while rhodopsin has an absorption spectrum independent of pH and is not affected by those reagents. This suggests that the link between chromophore and protein in retinochrome is more susceptible to the environment than in rhodopsin. On the other hand, evidence from circular dichroism measurements indicates that the chromophore of retinochrome is fixed to the protein part so that its rotation is restricted as in rhodopsin^{2,3}. Here we show that the absorption maximum of retinochrome is shifted to the shorter wavelength with increasing salt concentration.

Eyes were enucleated from fresh squids (*Todarodes pacificus*) under dim white light, frozen immediately, and stored at –20° C until used. For each preparation, we used about 40 eyes. They were thawed gradually at room temperature and bisected, after which the hemispheres containing the retinae were shaken in phosphate buffer (1/15 M, pH 6.8) in order to detach the outer segments of the visual cells and free the retinae

Fig. 1 Absorption spectra of retinochrome at pH 5.4 and 13° C. Curve 1 is the absorption spectrum of desalted retinochrome—digitonin solution. Next, solid NaCl (60 mg) was added to the solution (1 ml) and the pH was adjusted to 5.4 by addition of 0.6 μ l 1 N NaOH (curve 2). Curve 3 is the spectrum of the sample after desalting by dialysis.

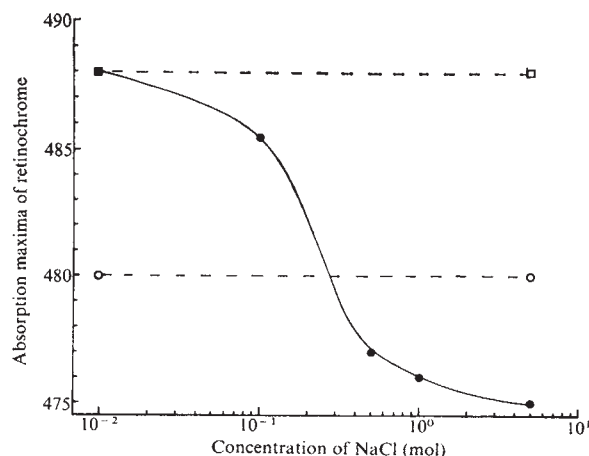
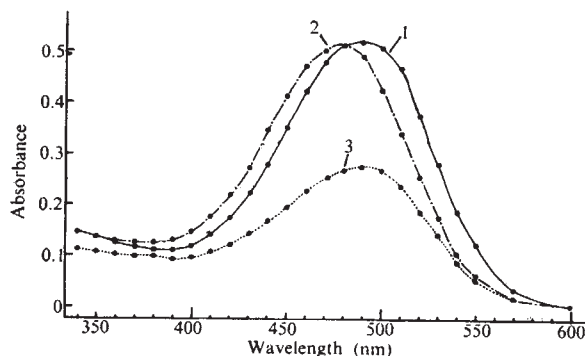


Fig. 2. Absorption maxima of retinochrome (●), rhodopsin (○) and acid-metarhodopsin (□) in various concentrations of NaCl at pH 5.4. The salt concentration of the samples was increased by addition of solid NaCl. When the sample became turbid by addition of NaCl, the absorption maximum was determined after centrifugation for 1 h at 28,000g at 4° C. Acid-metarhodopsin was prepared by converting rhodopsin to alkaline metarhodopsin by exposure to yellow light ($\lambda > 520$ nm) at pH 10, and then lowering the pH to 5.4.

containing the retinochrome. From the outer segment we extracted rhodopsin according to a method described previously². When retinochrome had been extracted from the outer segments together with rhodopsin, the two were separated by the chromatographic method described previously². Retinochrome was prepared from the outer segment-free retinae¹.

Ommochrome was eliminated from the extracts by passing them through a DEAE cellulose column (1 cm in diameter and 5 cm in height). The extract was placed on top of a column equilibrated with 0.02 M phosphate buffer (pH 6.8) and eluted with 0.02 M phosphate buffer containing 0.1% digitonin. As ommochrome was strongly adsorbed at the top of the column, eluates were free from ommochrome. The eluates were gathered, concentrated by a Sartorius membrane filter and then desalted by passage through a Sephadex G-25 column (1 cm in diameter and 20 cm in height), which was equilibrated with non-ionic 0.1% aqueous digitonin. We used the desalted retinochrome and rhodopsin samples to investigate the effect of salt on retinochrome and rhodopsin. Absorption spectra were measured with a 124 type Hitachi spectrophotometer at 13° C. Ionic strength was monitored with a conductivity meter (CD-35 M II, MS KiKi).

Figure 1 shows the absorption spectrum of retinochrome from 340–600 nm at pH 5.4. Retinochrome has an absorption peak at 488 nm in desalted digitonin solution (curve 1). When solid sodium chloride was added to this sample to a final concentration of 1 M, the absorption maximum was shifted to 478 nm (curve 2). At this salt concentration, no further change of the absorption spectrum was observed for at least 8 h at 13° C. When the sample was desalted by dialysis with a Sartorius membrane filter against distilled water for 5 h at 5° C, it again exhibited an absorption spectrum with the peak at 488 nm (curve 3). The absorbance at 488 nm was reduced to about half the initial value, because of the dilution of the sample during dialysis. This result shows that the change in the λ_{max} of retinochrome by salt is reversible.

Figure 2 shows the effects of salt concentration on the λ_{max} of retinochrome, rhodopsin and acid-metarhodopsin at pH 5.4. The λ_{max} of retinochrome shifted considerably at concentrations between 0.1 M and 0.5 M NaCl, and was at 475 nm in 5 M NaCl, while no shift of λ_{max} of rhodopsin and acid-metarhodopsin was observed. We further investigated the effect of various salts (NaCl, KCl, LiCl, MgCl₂ and CaCl₂) on the λ_{max} of retinochrome. All the salts so far examined caused the shift of the λ_{max} from 488 nm to a limit of 475 nm at pH 5.4

with increasing concentration. The concentration necessary for a 6-nm shift, about a half the maximum, was 0.03 M for CaCl_2 and MgCl_2 , 0.2 M for KCl and LiCl , and 0.4 M for NaCl .

Next, we examined the salt effect on the absorption spectrum of retinochrome in alkaline solution. When retinochrome was brought from pH 5.4 to pH 9.5 in desalted solutions, its λ_{max} shifted from 488 nm to 495 nm. At the same time, the absorbance at 495 nm became smaller than the initial absorbance at 488 nm and another peak appeared at 370 nm, as already reported^{1,2}. When solid sodium chloride was added to the alkaline solution to a final concentration of 1 M, no shift of either of the absorption peaks at 495 nm and 370 nm was observed. Figure 3 shows the effect of pH on λ_{max} of retinochrome in digitonin solutions containing 0.01 M and 1 M NaCl . The λ_{max} of retinochrome gradually changed from 484 nm at pH 4.5 to 495 nm at pH 9.3 in 0.01 M solution. In 1 M NaCl the λ_{max} was 476 nm at pH 4–5 and shifted to longer wavelengths above pH 6, reaching a limit of 495 nm at pH 9.3. This result indicates that the salt affects the pH dependency of the absorption peak of retinochrome.

It has been suggested that the λ_{max} of rhodopsin could be explained on the basis of a protonated Schiff base of retinal and amino group of opsin, for the λ_{max} of a protonated Schiff base tends to shift to the longer wavelengths, when the protonated portion $-\text{C}=\text{N}^+-$, is at a longer distance from a

negative counter anion, so that the net charge on the protonated Schiff base is more positive^{4,5}. We believe that the absorption peak of retinochrome is also due to a protonated Schiff base of retinal with the protein like that of rhodopsin. The following mechanism can therefore be considered for the dependence of λ_{max} of retinochrome on salt concentration. First, there is a direct action of the salt on the primary binding site, Schiff base linkage. Anions such as chloride will concentrate at the protonated portion of the Schiff base linkage and decrease its net positive charge. Although the chromophore binding site in retinochrome is probably more exposed than in rhodopsin, the shift in λ_{max} of retinochrome is not adequately explained by such a direct action of anions, because it depends on the species of cation, as described.

A second explanation involves the indirect actions of salts on retinochrome. It is well known that salts such as LiCl and CaCl_2 at concentrations above 1 M cause the denaturation of some proteins⁶. Furthermore, in the bleaching of cattle rhodopsin, an increase in ionic strength facilitates the conformational change in the protein moiety that corresponds to the conversion from Meta I to Meta II⁷. Salt may affect some of the dissociated amino acids residues in the protein parts resulting in conforma-

tional change in protein parts. Such a mechanism is also probable in the case of retinochrome. If the increase in salt concentration causes conformational changes of the protein moiety and thereby decreases the distance between the protonated Schiff base and its counter anions this could result in the blue shift of λ_{max} of retinochrome to shorter wavelengths. On the other hand, λ_{max} of cephalopod rhodopsin, metarhodopsin and other visual pigments are not affected by salt concentration. This suggests that the chromophore binding site in retinochrome is not so deeply embedded in the protein moiety as are those of the visual pigments and/or that the protein moiety surrounding the binding site is very different in retinochrome and in the visual pigments.

Further investigations on the binding site of the chromophore of retinochrome in comparison with that of rhodopsin and metarhodopsin may help to clarify the interrelationships between retinochrome and rhodopsin.

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Received June 21, 1974.

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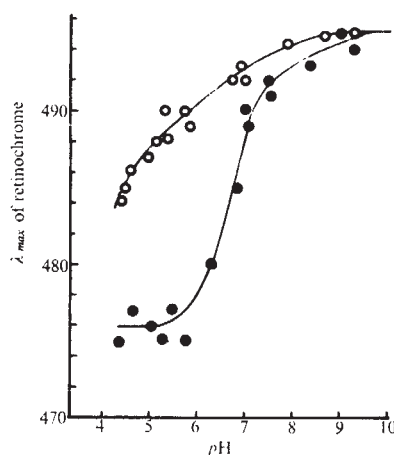
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Fig. 3 Dependence of λ_{max} of retinochrome on pH in digitonin solutions containing 0.01 M (○) and 1 M (●) NaCl . When the samples became turbid by the acidification or alkalisation, they were centrifuged 1 h at 28,000g at 4°C before measuring their absorption spectra.



Non-constant evolution rates in amino acid sequences of guinea pig, chinchilla and coypu pancreatic ribonucleases

THE primary structures of the pancreatic ribonucleases from cow¹, rat², pig^{3,4}, horse⁵, red deer and roe deer⁶, sheep⁷, goat⁸ and giraffe⁹ have been determined. From an analysis of these data we concluded that mammalian pancreatic ribonucleases have evolved with a rather constant evolution rate of about 1% substitution every 3 Myr (ref. 6). The validity of this conclusion, however, and possible exceptions have to be tested on a larger number of primary structures of mammalian ribonucleases.

As the amino acid sequences of the insulins from guinea pig and coypu differ markedly from those of other mammalian species^{10,11}, the amino acid sequences of other proteins from hystricomorph rodent species are of great interest. Here we describe the amino acid sequences of the pancreatic ribonucleases from the hystricomorph rodent species: guinea pig (*Cavia porcellus*), chinchilla (*Chinchilla brevicaudata*) and coypu (*Myocastor coypus*). Bartos and Uziel¹² described the isolation of two ribonuclease components from guinea pig pancreas. We have reproduced this finding and demonstrated that both components differ in amino acid sequence.

Ribonucleases were isolated by extraction of pancreatic

tissue with 0.125 M sulphuric acid, precipitation with acetone, ammonium sulphate fractionation, gel filtration on Sephadex G-25 and repeated chromatography on CM-cellulose (according to Åqvist and Anfinsen¹³ and with a sodium acetate gradient⁵, respectively). The amino acid sequences have been determined on tryptic digests of the aminoethylated proteins. The tryptic peptides have been positioned in the sequence by homology with other pancreatic ribonucleases and with each other. Full details of the isolation of the enzymes, several properties and the sequence determinations will be described elsewhere.

The coypu pancreas has one carbohydrate-containing ribonuclease component. From guinea pig pancreas two ribonucleases have been obtained, which not only differ in the presence (RNase B) or absence of carbohydrate (RNase A), but also in amino acid sequence. From chinchilla pancreas two carbohydrate-containing ribonuclease components have been obtained—one homogeneous and the other heterogeneous. The latter differs from the first in being more acidic and exhibits heterogeneity both in its carbohydrate moiety (glycopeptides both with and without sialic acid have been isolated) and in amino acid sequence

Table 1 Difference matrix of pancreatic ribonucleases

	Cow	Pig	Horse	Coypu	Chinchilla	Guinea pig A	Guinea pig B	Rat
Cow	—	20%	27%	27%	24%	25%	27%	34%
Pig	26	—	23%	25%	22%	27%	21%	35%
Horse	35	30	—	25%	21%	26%	28%	34%
Coypu	36	33	33	—	15%	24%	20%	40%
Chinchilla	32	29	28	19	—	21%	22%	36%
Guinea pig A	33	36	34	31	27	—	24%	39%
Guinea pig B	35	28	37	26½	28½	31	—	40%
Rat	44	46	45	52	47	51	52	—

Both the number and percentage differences are calculated from a hypothetical maximum chain length of 131 residues.

(probably glycine at position 32 has been partially substituted by aspartic acid).

The amino acid sequences of coypu, chinchilla and guinea pig ribonucleases A and B are shown in Fig. 1. Chinchilla and guinea pig ribonuclease A have a 'normal' chain length of 124 residues. Coypu and guinea pig ribonuclease B have additional amino acid residues at the C-terminus as has been found earlier in horse ribonuclease⁵.

The origin of both guinea pig ribonucleases must reside

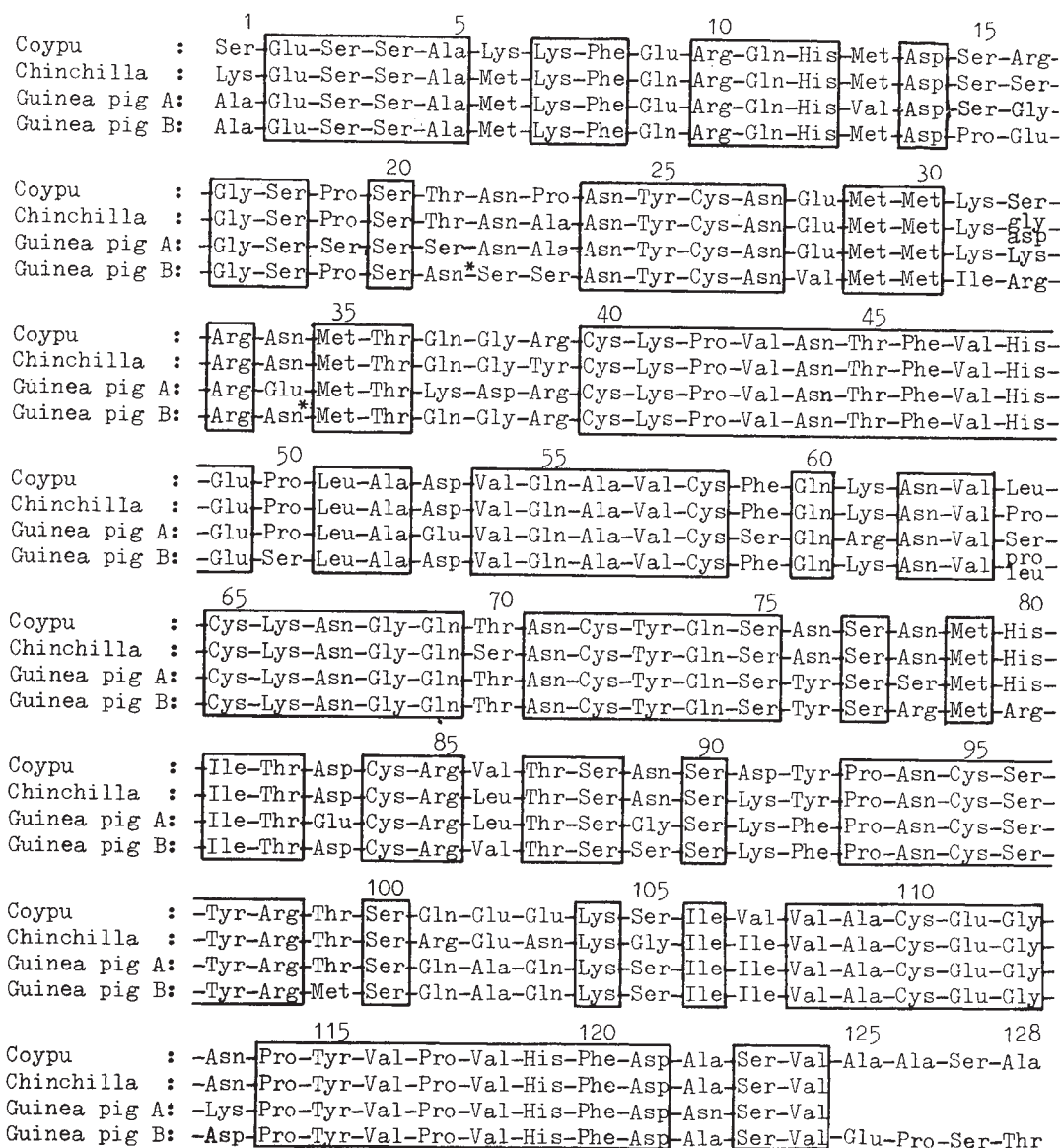


Fig. 1 Amino acid sequences of the ribonucleases of coypu, chinchilla and guinea pig. Identical residues in the four sequences are enclosed in blocks. Asn*, asparagine with carbohydrate.

Position	:	1	9	16	21	23	32	64	76	78	86	89	92	102	103	113
ANCESTOR HYSTRICOMORPH RODENTS	:	LYS	GLU	GLY	THR	ALA	ARG	PRO	ASN	SER	LEU	SER	TYR	ALA	GLU	ASN
ANCESTOR COYPU AND CHINCHILLA	:	LYS	GLU	ARG	THR	ALA	SER	PRO	ASN	ASN	LEU	ASN	TYR	GLU	GLU	ASN
Coypu	:	Ser	Glu	Arg	Thr	Pro	Ser	Leu	Asn	Asn	Val	Asn	Tyr	Glu	Glu	Asn
Chinchilla	:	Lys	Gln	Ser	Thr	Ala	gly asp	Pro	Asn	Asn	Leu	Asn	Tyr	Glu	Asn	Asn
ANCESTOR GUINEA PIG	:	ALA	GLU	GLY	SER	ALA	ARG	PRO	TYR	SER	LEU	SER	PHE	ALA	GLN	ASN
Guinea pig A	:	Ala	Glu	Gly	Ser	Ala	Lys	Ser	Tyr	Ser	Leu	Gly	Phe	Ala	Gln	Asp
Guinea pig B	:	Ala	Gln	Glu	Asn	Ser	Arg	pro leu	Tyr	Arg	Val	Ser	Phe	Ala	Gln	Lys

Fig. 2 Amino acid residues in the ribonucleases of coypu, chinchilla, guinea pig (A and B) and the hypothetical ancestors of chinchilla and coypu ribonuclease, of the two guinea pig ribonucleases and of the hystricomorph rodent ribonucleases, at the 15 positions where three or four different residues are found in the four ribonucleases occurring now. The ancestral residues have been derived from the sequence data in Fig. 1 and refs 1-9, and from the genetic code. Identical residues in coypu and chinchilla ribonuclease and in the two guinea pig ribonucleases are enclosed in blocks.

in a gene duplication. The many differences in amino acid sequence and also in other features make it unlikely that the two components represent alleles. The gene duplication may have occurred sometime before or after the divergence of the guinea pig from the other two hystricomorph rodent species investigated. In the first case we must assume that one of the two genes is a silent gene and does not come to expression in the other species or was lost again. In the other case the presence of two ribonuclease genes has been a novel acquisition in the line leading to the guinea pig, giving rise to new structural and functional possibilities which differ for the two gene products, resulting in an increased rate of acceptance of mutations. Here we provide evidence that the second possibility is more probable.

Table 1 is a difference matrix for the amino acid sequences of the ribonucleases from the hystricomorph rodents, cow, pig, horse and rat. Ribonucleases of species from different mammalian orders differ in about 30-35 positions (about 25%), with the exception of rat ribonuclease which differs in about 45-50 positions (about 35%) from all the others, including the hystricomorph rodent ribonucleases. Preliminary sequence information from the ribonuclease of the muskrat (a myomorph rodent species like the rat) indicates that the evolution rate of rat pancreatic ribonuclease has increased considerably after divergence of the cricetidae (including the muskrat) from the muridae (including the rat) (H. van Dijk, A. van den B., W. Gaastra and J. J. B., unpublished).

The data in Table 1 show that the four hystricomorph rodent ribonucleases form a group of related sequences. But as a group they do not show the deviating behaviour from the homologous proteins of other mammalian species as has been encountered before with the insulins of the same species^{10,11}.

It is difficult to reconstruct the evolutionary history of amino acid sequences from a difference matrix if the evolution rates have not been constant and equal in all lines¹⁴. Therefore, we prefer to use an approximation of the ancestral sequence method^{15,16} for this purpose.

Excluding the additional residues at the C-terminus in coypu and guinea pig B, the 124 amino acid positions can be divided into three groups. (1) 85 out of 124 (69%) positions are identical in the four sequences (these are the blocked sequences in Fig. 1) and are also an indication of the relatively close relationship between them (Table 1). (2) 24 positions differ in only one of the four sequences; the other three are identical in these positions. The distribution of these 24 deviating residues between the four

sequences is as follows (Fig. 1): three in coypu ribonuclease; four in chinchilla ribonuclease; ten in guinea pig ribonuclease A; seven in guinea pig ribonuclease B. These data point to an increased evolution rate in the two guinea pig ribonucleases as compared to the other two, but cannot provide us with the information necessary to deduce the evolutionary tree of the four sequences. (3) This goal must be accomplished with the data for the remaining 15 positions occupied by three or four different residues in the four sequences. The residues at these 15 positions are given separately in Fig. 2. There are strikingly close relationships between the ribonucleases from chinchilla and coypu (seven identities) and the two guinea pig ribonucleases (five identities). Chinchilla and guinea pig ribonuclease A correspond in two positions; other combinations correspond in a smaller number of positions.

These data are the basis of the evolutionary history of the four hystricomorph rodent ribonucleases shown in

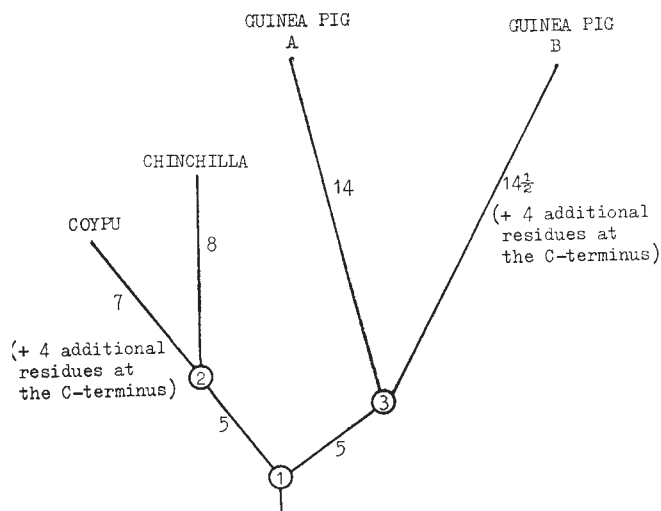


Fig. 3 Evolutionary tree of the hystricomorph rodent ribonucleases. Node 1, hypothetical ancestor of the hystricomorph rodent ribonucleases; node 2, hypothetical ancestor of the ribonucleases of coypu and chinchilla; node 3, hypothetical ancestor of ribonuclease A and B of guinea pig. The number of amino acid substitutions between two sequences are given next to the lines connecting those sequences. The lengths of these lines are drawn in proportion to these numbers. It has been assumed for simplicity that the 24 positions which differ in only one of the four sequences (group 3: see text) are occupied in the three ancestral sequences by the residues which occur in three of the four sequences.

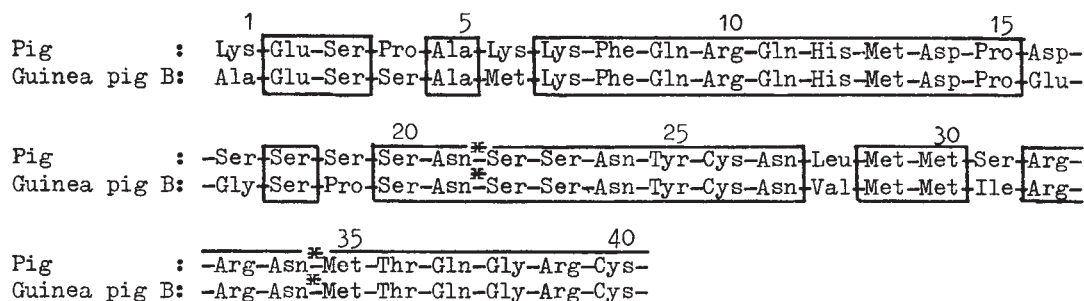


Fig. 4 Comparison of the N-terminal sequences of porcine and guinea pig ribonuclease B. Compare the similarities between these two sequences with the homologies in this part of the molecule of the hystricomorph rodent ribonucleases (Fig. 1) and between porcine and bovine ribonuclease³. Asn*, asparagine with carbohydrate.

Fig. 3. Coypu and chinchilla ribonuclease have evolved with a rather normal evolution rate. The ancestral guinea pig ribonuclease has branched off considerably earlier than the divergence between coypu and chinchilla ribonuclease. The next event which took place was a gene duplication resulting in two guinea pig ribonucleases, which have diverged from each other with a considerably increased evolution rate.

The amino acid sequence of the carbohydrate attachment site Asn-21 is identical in porcine and guinea pig ribonuclease B. It is evident from the difference matrix (Table 1) that there are more similarities in structure between these two proteins (both sequences differ at 24 positions, excluding the four additional residues at the C-terminus in guinea pig B), so they differ significantly less than other ribonucleases from different mammalian orders. These structural similarities are predominantly found in the N-terminal part of the sequence (Fig. 4), where, as well as many identities, two strikingly conservative substitutions are also found at position 16 (Glu/Asp) and 28 (Val/Leu). Perhaps the formation of identical carbohydrate attachment sites at Asn-21 has necessitated the development of more structural similarities than the Asn-Ser-Ser (21-23) sequence. A point in case may be the formation of a hydrophobic surface on the protein near the attachment site of the carbohydrate. Two remarkable substitutions in guinea pig B are the valine at position 28 and the isoleucine at position 31 instead of the charged residues glutamic acid and lysine, respectively, which occur at these positions in the ribonuclease of the other hystricomorph rodents. These two residues are at neighbouring sites at the surface of the molecule (as can be deduced from a model of bovine ribonuclease S) and it is very likely that they are in contact with the carbohydrate chain attached to Asn-21. In porcine ribonuclease the positions 28 and 31 are occupied by the non-charged residues leucine (hydrophobic like Val-28 in guinea pig B) and serine, respectively. Thus, the structural similarity between porcine and guinea pig ribonuclease B may be a case of structural convergence of two homologous proteins which have developed identical secondary characteristics.

We thank Mrs E. J. van den Hende-Timmer for technical assistance. We also thank the Streeklaboratorium voor de Volksgezondheid, Groningen, for collecting pancreatic tissue from guinea pigs and Bosch Internationale Chinchilla, Aalst, The Netherlands for collecting pancreatic tissue from chinchillas. Part of this work was carried out under the auspices of the Netherlands Foundation for Chemical Research and with financial aid from the Netherlands Organisation for the Advancement of Pure Research.

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Received August 22; revised November 12, 1974.

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A cis-dominant regulatory mutation affecting enzyme induction in the eukaryote *Aspergillus nidulans*

STUDIES on cis-acting regulatory mutants (for example, operator and promoter mutants) in bacteria have been of crucial importance in understanding gene regulation¹⁻⁴. Similar mutants in eukaryote systems have not been extensively studied although there have been two reports of probable operator constitutive mutants in yeast^{5,6}. An understanding of eukaryote genetic organisation and gene regulation requires that mutants affecting the regulation of adjacent genes of defined function be isolated and characterised. This communication describes a mutant of *Aspergillus nidulans* that is altered in a site closely linked to the structural gene (*amdS*) for acetamidase (E.C.3.5.1.4)^{7,8} and results in increased inducibility of this enzyme by acetamide. It is further shown that this mutation is cis-dominant in its effects.

In *Aspergillus nidulans*, lesions in the gene *areA* can result in inability to utilise a wide variety of nitrogen sources, including acetamide⁹. This gene is probably involved in regulation of enzyme synthesis by ammonium (ref. 9 and M.J.H., unpublished). Strains containing the mutation, *areA217*, grow very weakly on acetamide (and many other compounds) as the sole nitrogen source in the presence of glucose or acetate as sole carbon sources, but grow normally on acetamide as

the sole carbon source. The original mutant was isolated from a strain containing the *areA217* lesion as a N-methyl-N'-nitro-nitrosoguanidine-induced revertant which could grow strongly on medium containing acetate as the sole carbon source with acetamide as the sole nitrogen source.

Genetic analysis has shown that the mutant contains a lesion, provisionally designated *amd19*, which is closely linked to *amdS*, the structural gene for acetamidase, and that it retains the *areA217* mutation. An *areA*⁺, *amd19* derivative obtained by crossing to a wild type strain, has been used extensively in further analyses.

Table 1 Growth properties of *amdI* strains on acetamide and acrylamide

Relevant genotype	Acetamide*	Acrylamide*	Acetamidase†
<i>areA217</i> , <i>amd1</i> ⁺	—	—	+
<i>areA217</i> , <i>amd19</i>	+	—	++
<i>areA</i> ⁺ , <i>amd1</i> ⁺	+	±	++
<i>areA</i> ⁺ , <i>amd19</i>	++	±	++

Growth tests on solid medium at 37° C were carried out as described previously¹⁰. Growth symbols: ++, very strong growth; +, strong growth; ±, weak growth; —, background growth. Symbols on different media are not equivalent.

*Present as sole nitrogen sources (10 mM) in glucose-minimal medium.

†Present as sole carbon and nitrogen source (50 mM).

The *amd19* lesion results in increased growth on acetamide, either as a carbon or as a nitrogen source (Table 1), but does not affect growth on other nitrogen sources. Acrylamide is a non-inducing substrate for acetamidase¹¹. Therefore the finding that strains containing *amd19* do not grow better than *amd1*⁺ strains on acrylamide as the sole nitrogen source in glucose medium, indicated that inducibility of the acetamidase might be affected by *amd19*. The enzyme results shown in Table 2 support this proposal. Strains containing *amd19* have higher acetamidase activities than *amd1*⁺ strains under conditions of induction by acetamide but similar activities to *amd1*⁺ strains under non-induced conditions. Therefore *amd19* appears to result in increased acetamidase induction by acetamide.

Table 2 Acetamidase activities of *amdI* strains

Final growth conditions*		Relevant genotype			
Glucose	Acetamide	<i>areA217</i> , <i>amd1</i> ⁺	<i>areA217</i> , <i>amd19</i>	<i>areA</i> ⁺ , <i>amd1</i> ⁺	<i>areA</i> ⁺ , <i>amd19</i>
Present	Absent	0	0	14	13
Present	Present	0	22	21	34
Absent	Absent	35	43	35	39
Absent	Present	81	270	60	274

Mycelia for enzyme assays grown, extracted and assayed by methods described previously^{12,13}. Units of activity = nmol ammonium per min per mg protein.

*Mycelia grown for 16 h in glucose-10 mM ammonium medium and then transferred to these media for a further 4 h before harvesting.

To see if the effects of *amd19* are limited to the *amdS* gene in the *cis* position it was necessary to obtain strains of genotype *amd19*, *amdS*[−] (where *amdS*[−] represents a structural gene lesion) by isolating spontaneous mutants of *amd19* strains which are resistant to fluoroacetamide. This technique readily yields *amdS*[−] mutants with low acetamidase activities⁷. In this way a number of *amd19*, *amdS*[−] strains have been isolated. The presence of lesions in *amdS* was confirmed by complementation tests in heterokaryons and diploids with known *amdS*[−] mutants, as well as the finding of close linkage to known *amdS*[−] mutants. In the cases of the *amd19*, *amdS*[−] mutants reported here, the retention of the *amd19* lesion has been shown by the recovery of *amd19*, *amdS*⁺ recombinants in crosses. Heterozygous diploids with *amd19* in *cis* or in *trans* to *amdS*⁺ genes have been constructed and tested for acetamide utilisation and acetamidase activities (Table 3). The *amd19* lesion is *cis*-dominant to *amd1*⁺ in such diploids, with strong growth on acetamide and elevated acetamidase activities occurring only when *amd19* is *cis* to *amdS*⁺.

amd19 strains produce an acetamidase that is indistinguishable from the enzyme of *amd1*⁺ strains in electrophoretic

Table 3 *Cis* dominance of *amd19* in heterozygous diploids

Relevant <i>amd</i> genotype	Growth on acetamide*	Acetamidase activity†
I+S ⁺	+	60
I9S ⁺	++	274
I9S-91	—	3
I9S-106	—	4
I+S-11	—	3
I9S ⁺ /I+S ⁺	++	208
I9S ⁺ /I+S-11	++	200
I9S-91/I+S ⁺	+	37
I9S-106/I+S ⁺	+	30
I9S-91/I+S-11	—	0

*Acetamide (10 mM) in solid glucose-minimal medium. Symbols as for Table 1.

†Mycelia grown for 16 h in glucose-10 mM ammonium medium and then transferred to minimal medium containing 20 mM acetamide as the sole carbon and nitrogen source for 4 h before harvesting. Units as for Table 2.

mobility in starch gels, thermolability and substrate specificity (M.J.H., unpublished). This, together with higher enzyme levels found only under induction conditions, makes it likely that *amd19* affects acetamidase regulation rather than structure. The simplest hypothesis to account for this data is that *amd19* affects a regulatory site necessary for interaction with a regulatory molecule involved in induction by acetamide. This site is altered by the *amd19* mutation such that inducibility is greatly increased. Although a number of possible regulatory genes affecting the acetamidase have been found (refs 7-9, 11, 13 and M.J.H., unpublished), it is not yet clear which of these is involved in induction by acetamide. The hypothetical regulatory molecule could have a negative action (repressor) and in this case *amd19* would cause repressor binding to be weaker. Alternatively (and perhaps more likely) the regulatory molecule could have a positive action (activator), with *amd19* allowing a more efficient activation of enzyme synthesis. Studies on the effects of *amd19* on the general control mechanisms, such as ammonium repression and catabolite repression^{8,12,13}, which affect acetamidase, are in progress. These,

together with genetic fine structure analysis, should provide important information regarding the relationship between the regulatory sites influencing the activity of the adjacent structural gene.

Research supported by Australian Research Grants Committee. Technical assistance by Iole Barbieri and Jan Dickinson is gratefully acknowledged.

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Received September 6; revised November 18, 1974.

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Role of premeiotic replication in gene conversion

CURRENT molecular models of meiotic recombination have attempted to explain both classical reciprocal recombination (crossing over) and non-reciprocal intragenic recombination (gene conversion), with a single unified mechanism^{1,2}. An important assumption inherent in such models is that both types of recombination occur during the prophase following premeiotic chromosome replication. To date, however, only crossing over (chiasma) has been convincingly shown to occur during meiotic prophase³. Gene conversion, which has been most extensively studied in the ascomycete fungi⁴, has, for a number of cogent reasons⁵, also been assumed to occur during prophase, yet direct evidence supporting this critical point is lacking. In fact, in an important paper⁶, concerning recombination in yeast, evidence was presented indicating that intragenic recombination and premeiotic replication coincided, a finding which argues against much current speculation. To clarify this situation we simultaneously measured premeiotic replication and gene conversion in the yeast *Saccharomyces cerevisiae*, and examined the genetic consequences of inhibiting DNA synthesis with hydroxyurea (HU). We show that during the premeiotic S phase cells acquire a 'potential' for completing gene conversion, but that the actual formation of stable intragenic recombinants occurs after replication and depends on a period of postreplicative DNA synthesis.

In yeast, meiosis is induced by transferring logarithmically growing cells to nitrogen-free sporulation medium⁷. The kinetics of various meiotic events are shown for diploid strain 4579 (Fig. 1a). Since this strain is heteroallelic for a non-complementing pair of mutations at the *leucine-2* locus

$$\frac{leu2-1}{+} \quad + \quad \frac{+}{leu2-27}$$

gene conversion could be monitored by the appearance of prototrophic recombinants (+ +) on leucine-free selective medium. The *leu2-1* and 2-27 alleles were chosen because genetic analysis⁸ revealed that prototrophs arose by gene conversion rather than by reciprocal recombination. The recovery of prototrophs coincided rather closely with the period of premeiotic replication (6–15 h) (Fig. 1a). Data from seven experiments correlating the extent of DNA synthesis with the recovery of recombinants are given in Fig. 1b, clearly showing the close temporal relationship between the two events. Coincidence between replication and intragenic recombination was also seen in diploids heteroallelic for *arginine-4*, *leucine-1*, *uracil-3*, and *adenine-8*. Sporulation, which signals the completion of meiosis, extended within the population from 14–30 h.

We considered two alternative explanations for the temporal coincidence between replication and the recovery of gene convertants: (i) gene conversion occurs during premeiotic replication, or (ii) premeiotic replication coincides with 'commitment' of the cell to a developmental pathway which includes gene conversion, but the actual conversion event takes place later, during the postreplicative interval. The second alternative exists because of the indirect way in which recombination is measured; in practice, conversion is detected by counting prototrophic colonies which appear several days after meiotic cells are plated on selective medium. Thus the exact time of formation of a recombinant gene is obscured⁹; conceivably it could have been many hours after a 'committed' cell was first plated on selective medium.

To distinguish between the two alternatives we used hydroxyurea (HU), a specific reversible inhibitor of DNA synthesis¹⁰. We reasoned that if recombinants were actually generated

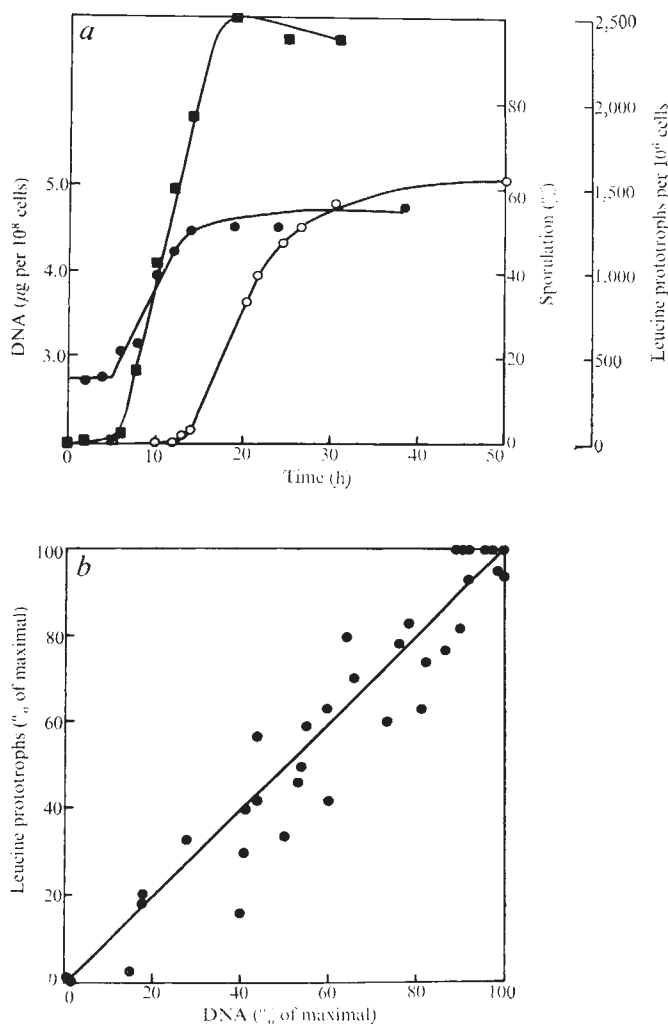


Fig. 1 Coincidence between recovery of gene convertants and replication during meiosis. Strain 4579 has the following genotype:

<i>his4</i>	<i>leu2-1</i>	+	<i>α</i>	<i>thr4</i>	<i>ade2</i>	<i>met2</i>	<i>ura3</i>
+	+	<i>leu2-27</i>	<i>a</i>	+	<i>ade2</i>	<i>met2</i>	<i>ura3</i>

Media and procedures used for growth and induction of meiosis (sporulation) were previously described¹¹, incubations were at 30°C. a, At zero time ($t = 0$) a growing culture was collected, washed, and suspended in acetate sporulation medium. At intervals, samples were removed to measure simultaneously: (i) DNA synthesis per cell (●), (ii) the frequency of leucine prototrophic recombinants (■), and (iii) the percentage of asci (○). DNA was determined using a semimicro modification of the procedure of Croes¹² (details to be published elsewhere). Leucine prototrophs were monitored by plating diluted culture samples on to leucine-free synthetic medium¹³. Ascus formation was determined by phase contrast microscopy. b, Data from seven independent experiments relating DNA synthesis and prototroph recovery were combined, as follows: for each experiment the total increase in DNA between $t = 2$ h and $t = 36$ h was set equal to 100%; similarly the maximal increase in prototrophs over this interval was also set to 100%. For each experimental point the percentage increase in DNA was plotted against the corresponding value for the increase in prototrophs.

during replication, then exposure to HU after replication was complete would not affect those recombinants already formed. In contrast, if gene conversion followed replication, then HU added to cells after replication might inhibit recombination by interfering with the localised, repair-type DNA synthesis which would be necessary for gene conversion¹¹.

In preliminary experiments we demonstrated that 100 mM HU inhibited both the initiation and continuation of DNA synthesis in meiotic cells. But, increases in mass, and protein

and RNA synthesis continued in essentially normal fashion (for up to 18 h) in cultures treated with HU. Thus, in meiotic cells as in mitotic cells¹⁰, HU selectivity inhibited DNA synthesis. The effects of HU on recombination are shown in Fig. 2; addition of the drug before replication completely blocked the appearance of meiotic recombinants, however, the low frequency of leucine prototrophs pre-existing in the population was not affected. When HU was added any time during replication, recombination was quickly halted; in addition, with continued incubation in HU there followed a rapid and extensive decline in the number of recombinants initially present at the time of drug addition. Eventually, the rapid loss of recombinants stopped, and their frequency reached a stable value which depended on the time of drug addition. As shown in Fig. 2 the decline in recombinants could not be attributed to a generalised toxic effect: viability even after prolonged drug exposure was > 95%. HU, added during replication, caused similar declines in the number of recombinants in diploids heteroallelic for unrelated loci (for example *arg-4*, *leu-1*, and *ura-3*). In no case could the decline be attributed to losses in viability.

Was the dramatic HU-induced decline in recombinant frequency associated with inhibition of ongoing chromosome replication (the premeiotic S phase), or with inhibition of postreplicative DNA synthesis? To answer this we had to determine the time when premeiotic S phase was completed in the asynchronous population of developing cells. Completion of replication was evaluated by blocking DNA synthesis (with HU) at various times and examining the ability of inhibited cells to complete meiosis, as judged by the formation of mature asci (Fig. 3). We considered sporulation a functional test for the completion of replication. Curve *a* of Fig. 3 depicts the final percentage of asci obtained in HU-inhibited cultures plotted against the time of drug addition. Presumably, curve *a* represents the kinetics of completion of replication in the population. Comparing curve *a* with the effects of HU on

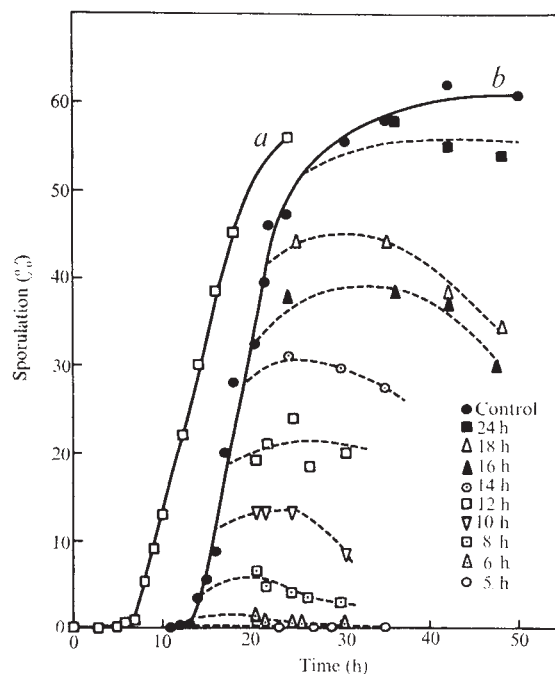


Fig. 3 Effects of hydroxyurea on sporulation. A meiotic culture of 4579 was established as in Fig. 1. Sporulation in this culture (● = control), is shown as curve *b* (solid line). At the times indicated on the figure (and at $t = 0, 3, 7$, and 9 h; detailed data not shown), portions of the control culture were transferred to separate flasks containing HU (final concentration = 100 mM). During subsequent incubation in HU, samples from each culture were removed, fixed in formaldehyde, and scored for percentage sporulation; these data are shown as a series of broken lines. Note that after prolonged incubation in HU the percentage of asci begins to decline. In curve *a* (□), the maximal level of sporulation reached in each of the HU-treated cultures is shown plotted against the time when the cells were first exposed to the drug.

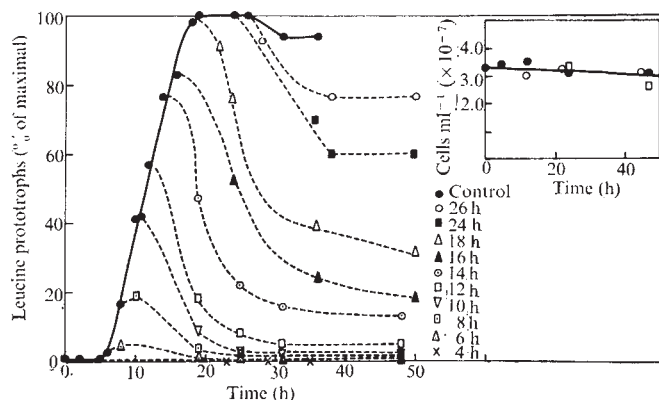


Fig. 2 Effects of hydroxyurea on meiotic gene conversion and viability. A meiotic culture of 4579 was established as in Fig. 1. Recovery of prototrophs in the control culture (●) is shown as a solid line. At the times indicated on the figure, portions of the control culture were transferred to separate flasks containing sufficient 2.5 M hydroxyurea to yield a final concentration of 100 mM. During subsequent incubation in HU, samples from each culture were plated to determine the frequency of remaining prototrophs; these values are shown as a series of broken lines. For simplicity, the maximal prototroph frequency in the control was set to 100%, and the absolute frequencies of prototrophs in the HU-treated cultures were converted to percentages of this value. Hydroxyurea (Squibb) was dissolved in H₂O, and filter sterilised just before use. Inset: effects of extended HU exposure on survival of meiotic cells. At $t = 5$ h and $t = 12$ h, portions of a meiotic culture (control) were transferred to flasks containing HU (final concentration = 100 mM). At intervals, samples from the control and HU-treated cultures were removed, sonicated briefly to disrupt clumps, diluted, and plated on complete medium¹⁴. Colonies were counted after 5 d. ●, Control; ○, HU at $t = 5$ h; □, HU at $t = 12$ h.

recombination (Fig. 2), revealed that the fraction of cells (relative to the control frequency) which completed replication was far in excess of the fraction of cells which formed stable (HU-resistant) recombinants. For example, at $t = 12$ h ~ 1/3 of the cells had completed replication, yet addition of HU at this time revealed that of the apparent recombinants (55%) only 5% were stable. This suggests that gene conversion cannot be consummated during the premeiotic S phase, but only later. In addition, the sensitivity of recombination to HU, in cells which have completed replication, indicates that successful gene conversion requires some postreplicative DNA synthesis.

Our conclusion that gene conversion occurred after replication depended on at least two assumptions: (i) that HU acted quickly to stop replication, and therefore, gave an accurate estimate for the time of completion of replication, and (ii) that ascus formation in HU is a valid functional test for the presence of a fully replicated genome.

To confirm independently that HU could provide an accurate estimate of the completion of replication (Fig. 3) in a population, we examined the consequences of blocking DNA synthesis using one of Hartwell's temperature-sensitive replication defective mutations¹². Strain Z-198 (Fig. 4) is homozygous for a thermal lesion in gene *cdc8*, which controls a step required for DNA polymerisation. During meiosis, a shift from a permissive temperature (23°C) to a restrictive temperature (34.5°C) immediately blocked replication. A culture of Z-198 was grown and transferred to sporulation medium at 23°C; at intervals duplicate samples were either shifted to 34.5°C, or put into HU at 23°C (Fig. 4). The final percentage sporulation attained plotted against the time of the HU or high-temperature challenge are compared in Fig. 4. Both treatments yielded virtually identical curves, and thus, provided the same estimate for the time of completion of replication in the population.

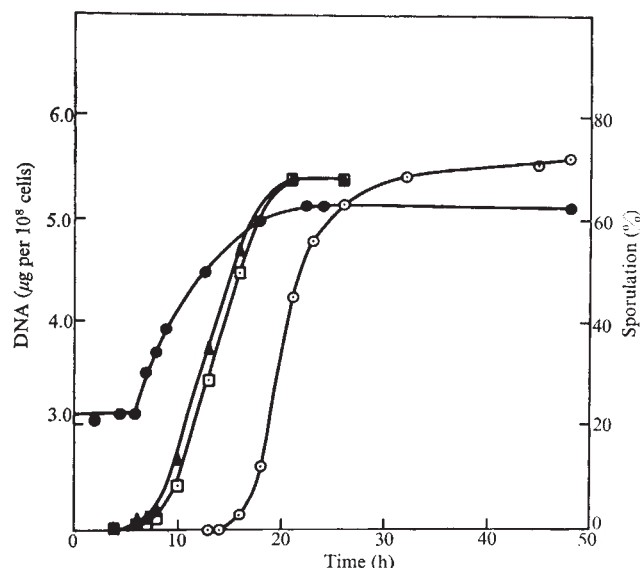


Fig. 4 Effects of hydroxyurea and restrictive temperature on sporulation of strain Z-198.

Thermosensitive strain Z-198 has the following genotype:

<i>cdc8-1</i>	<i>ura1</i>	<i>his4</i>	<i>leu2</i>	<i>α</i>	<i>thr4</i>	<i>ade2</i>	<i>tyr1</i>
<i>cdc8-1</i>	<i>ura1</i>	+	+	<i>a</i>	+	+	+
<i>his7</i>	<i>lys2</i>	+	+	<i>ade1</i>	+		
+	+	<i>met2</i>	<i>trp5</i>	<i>ade1</i>	<i>gal1</i>		

It was constructed by mating Hartwell's *cdc8-1* parent strain¹² to a thermoresistant haploid of opposite mating type (x1069-2D), and selecting for a thermosensitive mitotic segregant following a sublethal dose of ultraviolet irradiation¹⁸. Homozygosity for *cdc8-1* was confirmed by complementation tests¹³. A meiotic culture of Z-198 was established as in Fig. 1, except that growth and sporulation of the control culture were conducted at 23°C. Premeiotic DNA synthesis (●), and sporulation (○), were determined as in Fig. 1. At intervals during meiosis duplicate samples were removed from the control culture and transferred, either to a flask prewarmed and maintained at 34.5°C, or to a flask maintained at 23°C, but containing HU (100 mM). Sporulation was monitored for an extended period (48 h) in each of the HU and heat-treated cultures. The maximal percent sporulation reached in each culture is shown plotted against the time when the cultures were first exposed to the HU (▲) or the restrictive temperature (□).

Evidence that the asci formed in HU contained viable ascospores was obtained as follows: meiotic cells (of strain 4579) were exposed to HU, at $t = 12$ h, and allowed to complete sporulation in the presence of the drug. The resulting asci were dissected, and the individual spores tested for survival on complete medium¹³. Viability for the HU-treated asci (86%), was similar to that in mature asci from a control culture never exposed to the drug (90%). Thus asci formed in HU contained viable haploid cells, presumably originating by replication of the original diploid genome.

Our primary objective was to reconcile data which suggested that gene conversion might coincide with replication, and current molecular models which assume that conversion (as well as reciprocal recombination) takes place after replication, during meiotic prophase. We have shown that while 'potential' intragenic recombinants are recoverable during premeiotic replication, stable recombinants are only formed considerably after the premeiotic S phase. These results are entirely consistent with current models.

This work was supported by a grant and a Career Development Award (to R.R.) from the National Institutes of Health.

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Received August 22; revised October 10, 1974.

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Increased sensitivity of lymphocyte Δ^1 -pyrroline-5-carboxylate reductase to inhibition by proline with transformation

THE mitogen-induced transformation of resting lymphocytes to proliferating blast forms is associated with a marked increase in amino-acid uptake¹, protein synthesis² and enzyme activities³. There is little information, however, on the effect of transformation on enzymes of amino-acid metabolism. We therefore examined the biosynthetic and degradative enzymes of the non-essential amino acid, proline⁴, in resting and transformed lymphocytes. We found a selective increase of proline biosynthetic enzymes in association with transformation. It is interesting that transformation also produced qualitative changes in Δ^1 -pyrroline-5-carboxylate reductase, the enzyme which catalyses the committed step in proline synthesis.

Lymphocytes from normal human donors were prepared routinely by dextran sedimentation of heparinised whole blood or by separation of whole blood on Ficoll-Hypaque gradients. The collected leukocytes were then washed in balanced salt solution and cultured at a cell density of 0.5×10^6 mononuclear leukocytes per ml of RPMI 1640 medium supplemented with 10% autologous plasma, 4 mM glutamine, penicillin (50 U ml⁻¹) and streptomycin (50 μg ml⁻¹). In each experiment half the cells were cultured with phytohaemagglutinin (Burroughs Wellcome) at a concentration of 1 μg ml⁻¹ (optimum concentration of this preparation as determined by thymidine incorporation) and are referred to as PHA cells; the remaining cells were cultured without PHA and are referred to as control cells. After specified periods of culture, cells were collected by centrifugation, washed three times in balanced salt solution at 4°C and disrupted by sonication in 1 ml of buffer appropriate for each enzyme assay. Enzyme activities of the lymphocyte sonicates were determined by radioisotopic assay methods as previously described⁵⁻⁸. Protein was determined by the method of Lowry⁹. In all instances enzyme specific activity is expressed as nmol product per h per mg protein.

PHA transformation produced marked changes in the enzymes of proline biosynthesis (Fig. 1). Twelve hours after the addition of PHA the specific activity of the proline biosynthetic enzyme, Δ^1 -pyrroline-5-carboxylate reductase (PCA reductase), began to increase considerably. Seventy-two hours after the addition of PHA, the specific activities of the biosynthetic enzymes, ornithine- δ -transaminase (OTA) and PCA reductase, increased to levels at least fifteen times those in either cultured control cells or fresh peripheral lymphocytes. In contrast, the proline degradative enzymes did not follow this pattern. With 72 h of PHA-stimulated mitogenesis, the specific activity of PCA dehydrogenase remained unchanged while proline oxidase activity remained undetectable in either sonicates or mitochondrial preparations. For proline oxidase we used a radioisotopic method able to detect less than 5 pmol of product. Thus, only the biosynthetic enzymes of proline metabolism are increased with lymphocyte activation.

The marked increase in PCA reductase was of special interest to us. We showed previously that PCA reductase exists in two forms: one sensitive and one insensitive to feedback inhibition by proline¹⁰. Enzyme obtained from resting cells in animal tissues (rat liver, brain, kidney) is relatively insensitive to proline inhibition with less than 50% inhibition at 10^{-2} M proline. In contrast PCA reductase from cells growing in culture (human fibroblasts, Chinese hamster ovary and lung cells and rat liver cells) is always markedly sensitive to proline inhibition with 50% inhibition at 2×10^{-4} M proline. Thus we considered whether the increase in PCA reductase in the transformed lymphocyte would be accompanied by a change in the proline sensitivity of the enzyme. Figure 2 shows the proline inhibition

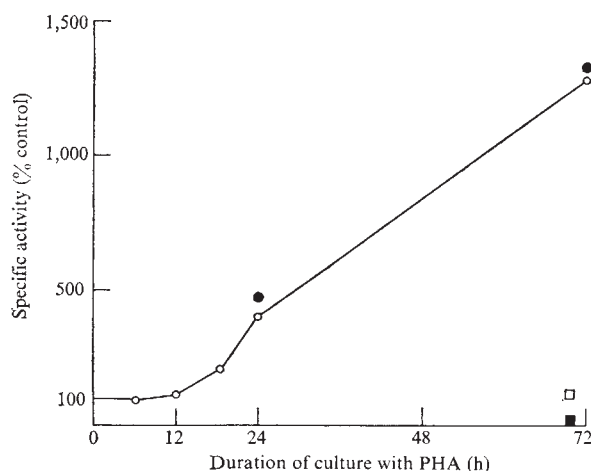


Fig. 1 Effect of phytohaemagglutinin (PHA) transformation on the enzymes of proline biosynthesis and degradation. Data are expressed as percentage change over control cells incubated without PHA. Each point represents the mean of at least three determinations performed on extracts from three separate cultures. PCA reductase (○); ornithine transaminase (●); PCA dehydrogenase (□); proline oxidase (■).

of PCA reductase from control and PHA cells after culture for 72 h. The control cell enzyme was relatively insensitive to proline being inhibited only about 30% by 10^{-3} M proline. The same curve of proline inhibition was obtained when either fresh peripheral lymphocytes or cells purified to greater than 99% lymphocytes were the enzyme source. In contrast, the PHA cell enzyme was markedly more proline-sensitive, with 50% inhibition by 2×10^{-4} M proline and 85% inhibition by 10^{-3} M proline. Clearly in addition to a 15-fold increase in specific activity with lymphocyte transformation, PCA reductase became markedly more sensitive to inhibition by proline. The slight inhibition of the control enzyme by proline may have resulted from the presence of a small amount of the proline-sensitive form of the enzyme in resting lymphocytes or from the presence of 1–2% activated lymphocytes in the control cell population.

The marked difference in proline sensitivity of PCA reductase

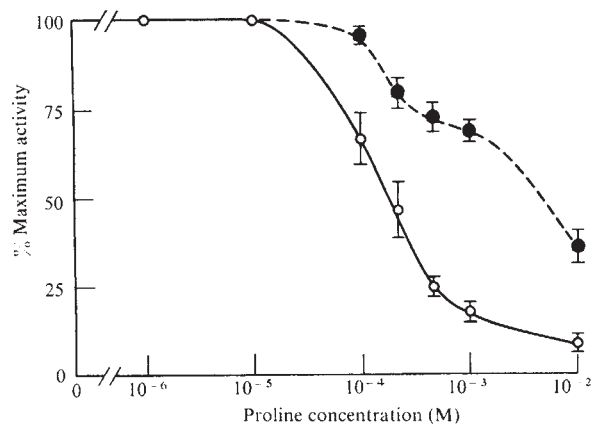


Fig. 2 Proline inhibition of PCA reductase. PCA reductase activities in extracts of 72-h PHA cells (○) and 72-h control cells (●) were assayed with various concentrations of proline. The assay mixture included Δ^1 -pyrroline-5-carboxylate, 0.045 mM (in the L configuration); NADH, 0.68 mM; phosphate buffer 0.1 M, pH 6.8, and 20 μ g of extract protein. The mixture was incubated at 37° C for 10 min. Each point represents the mean $\pm$ s.e.m. of three determinations performed on extracts from three cultures.

in 72-h transformed lymphocytes may represent the selective synthesis of proline sensitive PCA reductase. In support of this hypothesis, we found that the time course of change in sensitivity to inhibition by proline paralleled the increase in PCA reductase activity, both showing a 12-h lag after addition of PHA. Furthermore, the total increase in PCA reductase specific activity with transformation represents an increase only in the proline-sensitive activity. The proline-insensitive activity (activity in the presence of 1×10^{-3} M proline) did not change significantly during 72 h of PHA transformation.

If the observed 12-h lag was related to the synthesis of mRNA coding for the proline-sensitive reductase and subsequent accumulation of the new enzyme, we should have been able to block the appearance of the enzyme with inhibitors of RNA and protein synthesis. When either actinomycin D (5 μ g ml⁻¹) or cycloheximide (0.05 mM) was added to PHA cells at time zero and cells were collected 18 h later, both the increase in PCA reductase activity and the appearance of proline sensitivity were blocked (Fig. 3). The PCA reductase activity of control cells was unaltered by a comparable exposure to inhibitors. Furthermore, actinomycin D added 8 h after addition of PHA in an otherwise similar experiment (data not shown) did not prevent increases in either PCA reductase activity or sensitivity to inhibition by proline. Therefore, these alterations in PCA reductase activity require both RNA and protein synthesis.

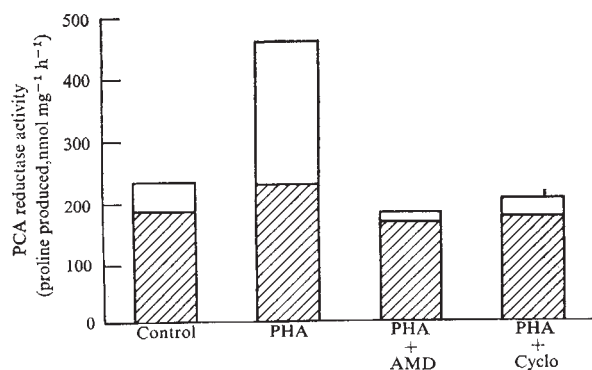


Fig. 3 The effect of actinomycin D and cycloheximide on PHA-induced increases in PCA reductase. Lymphocytes were cultured for 18 h with PHA, PHA plus actinomycin D, (5 μ g ml⁻¹) or PHA plus cycloheximide (0.05 mM). The bars represent total enzyme activity whereas the cross-hatched areas represent activities in the presence of 10^{-3} M proline. Data represent the mean of at least three determinations performed on extracts from three cultures.

The most straightforward explanation of these data would involve the preferential synthesis of a proline-inhibitable isozyme of PCA reductase in association with the change from resting to actively growing lymphocytes. This hypothesis is consistent with our work demonstrating the occurrence of proline-insensitive PCA reductase in resting cells obtained from animal tissues and proline-inhibitable reductase obtained from cells adapted for rapid growth in tissue culture¹⁰. Confirmation of this hypothesis will require direct demonstration of two forms of PCA reductase.

The accumulation of a proline-sensitive PCA reductase is beneficial to the activated lymphocyte in two ways. The proline synthetic capability is increased to meet the demands of increased protein synthesis and the proline sensitivity of this enzyme makes proline synthesis subject to negative feedback control. Thus, in the activated lymphocyte if exogenous proline were adequate to meet the demands of increased protein synthesis, PCA reductase would be inhibited, sparing proline precursors for use elsewhere in the cell.

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Human immunoglobulins with a1 allotypic determinants of rabbit immunoglobulin heavy chains

ALLOTYPIC genetic variations in the variable (V) part of immunoglobulin (Ig) chains have been clearly identified only in the domestic rabbit. Three allotypic specificities, a1, a2 and a3, controlled by allelic genes, *a*¹, *a*² and *a*³, at the *a* locus, are present on the variable region of heavy chains of all classes (IgM, IgG, IgA and IgE)¹⁻³. These allotypic specificities reflect multiple amino acid differences in the V region of Ig heavy chains⁴.

Subgroups also differ in the amino acid sequence in the V region. The heavy chains bearing the specificities controlled by the *a* locus represent a heavy chain subgroup designated V_Ha which accounts for 70-90% of the Ig heavy chains in the rabbit. The N-terminal amino acid sequence of pooled Ig heavy chains of the V_HIII subgroup from each of nine different species (including cat, dog, human, mouse, rat, guinea pig, mink, seal and sea lion), revealed striking similarities⁵. With one exception (cat), the sequence in any species did not differ from the sequence in any other species by more than four of the N-terminal 24 amino-acid residues⁵. Also, Rodkey and

Hansen⁶ found that some of the a2 and a3 allotypic specificities are present on the Fab fragment of IgG from cottontails and jack rabbits which are species closely related to the domestic rabbit. These observations led us to investigate whether anti-allotypic antisera, directed against the allotypic specificities present on the V part of rabbit heavy chains would react with human immunoglobulins. This report deals with the reaction of anti-a1 anti-allotype antiserum with human IgG (Hu IgG).

Pooled Hu IgG (FR II, Pentex, Kankakee, Illinois) was labelled with ¹²⁵I (ref. 7) and tested for the presence of the a1, a2 and a3 determinants by an indirect precipitation assay. Rabbit anti-a1, anti-a2 or anti-a3 antiserum was added to 0.1 µg ¹²⁵I-IgG; after 30 min at 37° C, goat anti-rabbit IgG antiserum (previously adsorbed with a Sepharose immunosorbent containing Hu IgG) was added to precipitate the complexes formed by the reaction of the anti-allotype antiserum and the ¹²⁵I-Hu IgG. After 24 h, the resultant precipitate

Table 1 Percentage precipitation of ¹²⁵I-labelled rabbit IgG or Fab_γ by rabbit anti-a1 antibody eluted from a Sepharose immunosorbent containing human IgG

Rabbit	Allotypes	¹²⁵ I-antigen	Radioactivity precipitated (%)
H209-3	a1,b5	IgG	85
		Fab _γ *	67
2L280-3	a1,b5	Fab _γ	75
F280-3	a1,b5	IgG	81
		Fab _γ	62
L244-1	a1,b5	IgG	89
		Fab _γ	68
F83	a2,b5	IgG	4
L16-1	a2,b5	IgG	5
03485	a3,b5	IgG	3

*The Fab_γ fragments were prepared by papain digestion of IgG followed by CM-cellulose chromatography as described by Porter¹³; the Fab I fragments were used as the antigens.

was washed, the radioactivity in the supernatant fluids and in the precipitates was determined and the percentage radioactivity precipitated was calculated⁸. Anti-a1 precipitated 71% of the radioactivity of the Hu IgG whereas anti-a2 and anti-a3 precipitated less than 5% of the radioactivity.

Those anti-a1 antibody molecules which reacted with Hu IgG were isolated from a Sepharose immunosorbent column containing pooled Hu IgG⁹. The anti-a1 antibody, reacting with Hu IgG, was used for the analysis of Hu IgG samples.

Hu IgG was isolated from serum samples of nine individuals by a combination of salt precipitation with Na₂SO₄ and DEAE-cellulose chromatography^{10,11}. The purified IgG samples were labelled with ¹²⁵I (ref. 7) and were shown to be more than 95% Ig by quantitative precipitation with rabbit anti-Hu IgG. These labelled IgG samples were reacted with the specifically purified anti-a1 by the quantitative radioprecipitation assay described above; from 45 to 70% (average, 61%) of the radioactivity was precipitated. In controls where normal (unimmunised) rabbit serum was substituted, for anti-a1 less than 9% of the radioactivity was precipitated.

To determine if the a1 specificity present on Hu IgG was a genetic variant or was present in all individuals, plasma samples from 270 individuals were tested for the presence of a1 specificities by a quantitative inhibition of precipitation assay¹². The 270 plasma samples were obtained from the clinical laboratories of our University Hospital and 5γ of a plasma sample was added to a tube containing 0.05 µg ¹²⁵I-Hu IgG (from an individual). An amount of anti-a1 that was slightly less than that needed to precipitate all of the ¹²⁵I-Hu IgG was added and the tubes were incubated for 30 min at 37° C; then, goat anti-rabbit IgG was added to precipitate the anti-a1 and any Hu IgG bound to the anti-a1. The percentage radioactivity precipitated was determined and the percentage in-

Table 2 Percent precipitation of ^{125}I -labelled human Fab_γ and F(ab')_γ fragments by anti-a1 anti-allotype antibody, anti-Hu IgG antiserum and anti-Hu Fc_γ antiserum*

Person	Labelled fragment	Precipitation by antibody to:		
		a1 %	Hu IgG %	Hu Fc_γ %
WJH	F(ab')_γ	36	79	1
UNK	Fab_γ	40	90	3

*Anti-Hu IgG has antibodies directed toward light chains and heavy chains. Anti-Hu IgG was made specific for determinants on the Fc part of IgG by passing the antiserum through a Sepharose immunosorbent column containing F(ab')_γ fragments of normal pooled human IgG

hibition was then calculated for each plasma sample. All 270 plasma samples completely inhibited the reaction of anti-a1 with Hu IgG; thus, all of the human sera tested possessed the a1 specificity.

That the specifically purified anti-a1 still reacted with the a1 determinants of the rabbit was shown by its ability to react with most (81–89%) of the IgG molecules as well as with the Fab_γ fragment obtained from rabbits with the genotype $a^1a^1b^5b^5$ (b^1 and b^5 are allelic genes at the b locus; these genes control the synthesis of rabbit b4 and b5 light chains, respectively). The purified anti-a1 did not precipitate the IgG molecules obtained from two $a^2a^2b^5b^5$ rabbits nor from one $a^3a^3b^5b^5$ rabbit (Table 1). These assays were done by the quantitative precipitation analysis as described above except that anti-b4, rather than goat anti-rabbit IgG, was used as the indirect reagent (the anti-a1 had been prepared in a b^1b^1 rabbit thus permitting the use of anti-b4 as an indirect reagent).

Human Fab_γ fragment was prepared by papain digestion¹⁴ of Hu IgG obtained from one individual (U.N.K.) and F(ab')_γ fragment was prepared by pepsin digestion¹⁵ of Hu IgG obtained from another individual (W.J.H.). Analysis of these fragments by the radioprecipitation assay revealed that the a1 determinant is present on the Fab_γ (40%) and the F(ab')_γ (36%) fragments (Table 2) as well as on the intact IgG (47%) of W.J.H. That the F(ab')_γ and the Fab_γ fragments were not contaminated with Fc fragments is shown by the observation that anti- Fc_γ did not react with the F(ab')_γ nor with the Fab_γ fragments, that is, less than 5% of the radioactivity was precipitated (Table 2).

Not all anti-a1 antisera reacted with Hu IgG. Of the anti-a1 antisera obtained from 5 different rabbits (E208-3, K158-3, 53923, H207-2, and J314-1) only E208-3 and K158-3 reacted with Hu IgG. The explanation for this observation is currently not clear. As not all anti-a1 antisera reacted with Hu IgG, we have recently examined several different anti-a2 and anti-a3 antisera and have indeed found an anti-a2 and an anti-a3 antiserum which react with Hu IgG.

In conclusion, we have shown that Hu IgG molecules have at least one antigenic determinant of the rabbit a1 specificity. This a1 determinant in man was present in all 270 individuals tested, was found on at least 60% of the IgG molecules and was localised to the Fab fragment. Population studies with anti-a1 as well as with anti-a2 and anti-a3 purified rabbit antibodies reacting with human IgG should resolve the genetic basis for these determinants in man and indicate whether they are isotypic or allotypic antigenic determinants, that is present in all individuals or segregated among the population as in rabbits. Studies with myeloma proteins of different classes and subgroups should be useful for the precise localisation and characterisation of these determinants in human immunoglobulins.

This work was supported in part by National Institutes of Health grants. K.L.K. is the recipient of an NIH Research Career Development Award.

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Received July 4; revised November 18, 1974.

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Uptake of environmental antigens by the bursa of Fabricius

THE bursa of Fabricius of birds has an essential role as a central lymphoid organ for the differentiation of B lymphocytes^{1,2}. In addition, the bursa harbours immunocompetent B cells³ which are capable of local antibody production. Haemolytic plaque-forming cells have been observed in the bursa after introducing sheep red blood cells (SRBC) into the bursal duct⁴. Furthermore, epithelial cells of the bursal follicles have been shown to transport ferritin and India ink into the intercellular spaces of the underlying lymphoid tissue when the material has been introduced into the bursal lumen⁵. The bursal lumen opens through the bursal duct to the most caudal of the three cloacal chambers, the proctodeum, which is of ectodermal origin^{6,7}. The proctodeum leads to the outside through the anus and is closed externally by a strong sphincter muscle. The vent is surrounded on the outside by upper and lower anal lips. At the other end, the proctodeum opens into urodeum leading to the coprodeum and these form the cranial parts of the cloaca (Fig. 1a).

During surgical bursectomy, we observed that the anal lips of a chicken make peristaltic movements as if sucking something into the cloaca. When a liquid or suspension was applied to the external parts of the anus, it was rapidly taken inside by these movements. We hypothesised that at least part of the sucked material could be taken into the bursa and that this phenomenon probably serves as a trapping mechanism for environmental antigens. To test the hypothesis we examined the macroscopic and microscopic fate of material applied to the anal lips, and whether antigen administration by this mechanism leads to an antibody production.

We placed 0.05–0.1 ml volumes of barium sulphate on the anal lips of 6-week-old White Leghorn chicks, and visualised the intake by X-ray pictures. It seemed that this contrast medium was actively sucked through the proctodeum and the bursal duct into the bursa. In all, about 2 ml of the medium was taken into the bursa in 3 min (Fig. 1b–e). Only negligible amounts of the contrast medium appeared in the urodeum and coprodeum. When applying the medium for the X-ray pictures presented here, the chicks were kept in a position with the anus upwards. In this position, most of the contrast medium was evacuated normally and the same results were obtained when the medium was applied to the anal lips of chicks in the normal position—even though this was technically more difficult.

To study the microscopic distribution of the material sucked by the bursa, 0.1–0.5 ml of India ink (Pelikan 17 noir, Günther

Wagner, Germany, dialysed overnight against physiological saline) was applied to the anal lips of 4-week-old chicks as described above. The chicks were anaesthetised with ether, and the bursae removed at appropriate intervals for microscopic examination. Two minutes after the application, no carbon was observed in the bursal tissues; 15 min after the application, some fine carbon particles occurred superficially in the follicle-associated epithelium and after 2 h the tracer had spread throughout the follicle-associated epithelium (Fig. 2a). The cylindrical epithelium lining the bursal lumen and not associated with the follicles was almost always free of the carbon. Six hours after the application, fine and coarse carbon granules were observed in the central part of the follicular medulla (Fig. 2b). These findings demonstrate that material available on the anal lips makes intimate contact with the lymphoid cells in the bursa so that antigenic sensitisation by this route is possible.

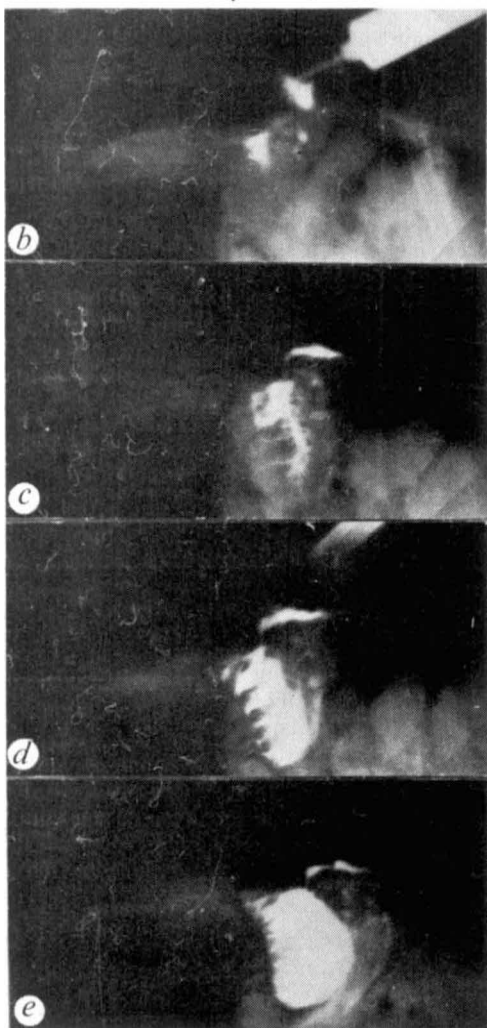
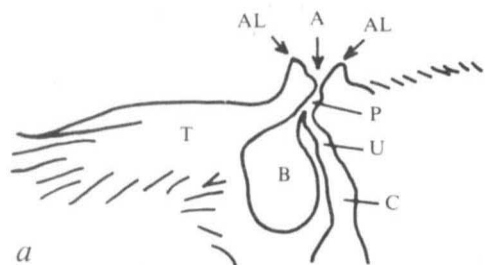


Fig. 1 *a*, Schematic presentation of the three cloacal chambers and the bursa of Fabricius in the chicken. A, anus; AL, anal lips; P, proctodeum; U, urodeum; C, coprodeum; B, bursa of Fabricius; T, tail. *b-e*, X-ray visualisation of barium sulphate applied on the anal lips. About 2 ml of the medium was sucked into the bursa in 3 min. Only negligible amounts appeared in the urodeum and coprodeum.

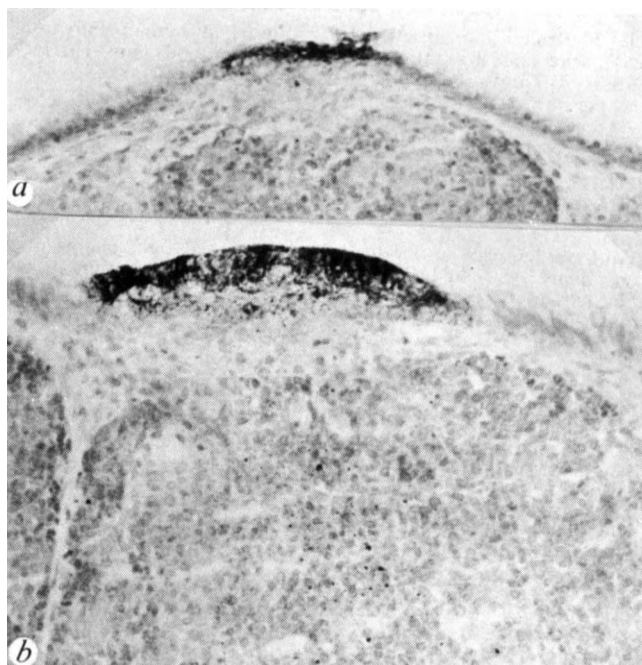


Fig. 2 Bursal sections after application of dialysed India ink on the anal lips. *a*, 2 h after the application, carbon granules appear in the follicle-associated epithelium while the follicle is free of carbon; *b*, after 6 h, carbon has spread more widely and deeply into the follicle-associated epithelium, and carbon granules are observed also in the medulla of the follicle. Weak methylgreen nuclear staining ($\times 256$).

To examine whether the antigenic challenge through the bursa leads naturally to antibody production, 10^8 killed *Brucella abortus* organisms and 10^{10} SRBC in 0.1 ml of physiological saline were applied to the anal lips of 4-week-old chicks as in the experiments described previously. The procedure was repeated on six consecutive days. Blood samples from a wing vein were collected on the day after the final antigen administration. High serum antibody titres against a thymus-independent antigen³, *Brucella*, were observed, whereas serum antibody titres against the thymus-dependent SRBC were close to those found in unimmunised controls (Table 1). We interpret these findings to indicate that antigenic challenge *per bursam* leads to an effective antibody formation if no T-B-cell cooperation is required. This interpretation is supported by the vigorous antibody formation by transferred bursa cells against *Brucella* but not against SRBC in T and B-cell deficient recipients³.

On the basis of these findings, it is apparent that the immunological function of the bursa of Fabricius is not restricted to its inductive role in the B-cell differentiation but that it also has an important peripheral function. Furthermore, it is apparent that this peripheral function does not serve only local antibody production but leads to significant antibody titres in the blood. It remains to be established whether this antibody formation is restricted to that against thymus-independent antigens and whether it leads to formation of antibodies of different immuno-

Table 1 Serum antibody titres (mean $\pm$ s.e.m.) after antigen application on the anal lips

Antigen*	No. of chickens	Log ₂ antibody titre† SRBC	Log ₂ antibody titre† <i>Brucella</i>
SRBC + <i>Brucella</i>	9	0.7 $\pm$ 0.2	6.8 $\pm$ 1.0
Saline	10	0.1 $\pm$ 0.1	0.4 $\pm$ 0.2

* 10^8 killed *Brucella abortus* organisms and 10^{10} SRBC in 0.1 ml were applied to the anal lips of 4-week-old chicks on six consecutive days.

†Serum antibody titres were determined from samples taken on the day following the final antigenic challenge; a microagglutination technique was used².

globulin classes in the same way as obtained after parenteral immunisation. It is also conceivable that constant presentation of environmental antigens in the newly-hatched period to the cells proliferating in the bursa leads to antigen-generated antibody diversity^{8,9}.

In conclusion, we suggest that a major function of bursa of Fabricius, in addition to its role as a central lymphoid organ, is to provide a chance for an early antigenic challenge by environmental antigens. The sucking function described here ideally serves this purpose.

This research was supported by grants from the Academy of Finland and the Sigrid Jusélius Foundation, and is pursuant to a contract with the National Cancer Institute, Bethesda, Maryland.

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Ly antigens as markers for functionally distinct subpopulations of thymus-derived lymphocytes of the mouse

THYMUS-DERIVED lymphocytes (T cells) have several functions¹⁻³, and in some instances it appears that two subpopulations of T cells interact to amplify their response^{4,5}. But recognition of the diverse functions and interactions of T cells has not been matched by the development of techniques for identifying and separating the different T-cell subpopulations involved. The ability to do so would greatly aid study of the many roles of T cells. We report here experiments with mice that show that antisera to different Ly alloantigens can identify functionally-distinct subpopulations of T cells.

The *Ly-1* and *Ly-2* loci (chromosomes 19 and 6) specify alloantigens expressed exclusively and invariably on mouse T cells⁶. Each locus has alternative alleles that determine the T-cell surface antigens *Ly-1.1* and *Ly-1.2*, and *Ly-2.1* and *Ly-2.2*, respectively.

The four corresponding Ly antisera were produced by alloimmunisation with T cells as described⁷ and were absorbed with syngeneic thymocytes, if necessary, to remove thymocyte auto-antibody⁸. The mice used were of the B6 strain (*Ly-1.2*; *Ly-2.2*), and of B6 congenic partner strains with phenotypes *B6/Ly-1.1* and *B6/Ly-2.1*.

Table 1 shows the proportions of peripheral lymphocytes, and of cortisone-resistant thymocytes (CRT), lysed by optimal concentrations of *Thy-1* (formerly θ) antiserum,

and by these Ly antisera in the cytotoxicity assay with rabbit complement (C). Ly antigens are present only on T cells, and we shall assume that all T cells are *Thy-1*⁺ (that is they carry *Thy-1* antigen); we have therefore expressed the results in Table 1 as proportions of T cells (*Thy-1*⁺ cells) lysed by each Ly antiserum (% T) as well as proportions of the total population (abs.=absolute).

The higher proportion of cells lysed by anti-*Ly-1.2* serum compared with anti-*Ly-2.2* serum was highly reproducible (Table 1) and may signify that there are more *Ly-1.2*⁺ T cells than *Ly-2.2*⁺ T cells among the peripheral T-cell population of B6 mice. This difference could also be explained, however, by differences in the constitution of the particular *Ly-1.2* and *Ly-2.2* antisera used (for example by different proportions of C-fixing antibody compared with other classes of antibody) and therefore more evidence is required on this point.

To determine whether T cells with different functions could be distinguished by characteristic Ly phenotypes, three functional assays for T cells were performed with cell populations pretreated with each Ly antiserum and C under essentially the same conditions as those of the C-dependent cytotoxicity assay⁹. We assayed (a) helper function of spleen cells from mice primed with sheep red blood cells (SRBC); (b) cell-mediated cytotoxicity (CMC) of non-adherent peri-

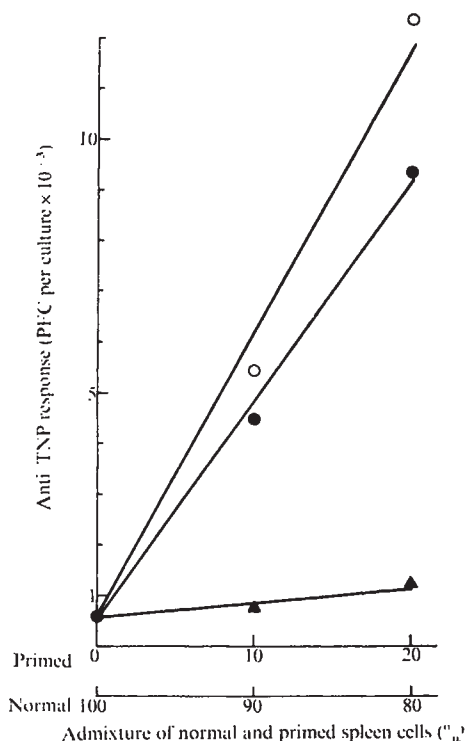


Fig. 1 Effect of pretreatment with different anti-Ly sera and C on the helper function of spleen cells. Three mice were injected intravenously with 0.1 ml of 0.01% (v/v) SRBC. After 4 d their spleen cells were pooled and pretreated with Ly antisera and C (see below). Cultures were prepared containing these pretreated primed cells together with unprimed cells in various proportions (as indicated), maintaining a constant total 1×10^7 cells per culture. The cultures were sensitised with trinitrophenol (TNP)-conjugated SRBC and assayed 4 d later for TNP-specific PFC^{11,12}. For pretreatment, equal volumes of cells (5×10^6 ml⁻¹); antiserum (1/20) and complement (rabbit serum preabsorbed with mouse cells in the presence of EDTA) (1/6) were incubated in 5% CO₂ in air for 45 min at 37°C. These are the standard conditions for the C-dependent cytotoxicity assay⁹ (see Table 1). The diluent was Earle's balanced salt solution with 2.5% foetal bovine serum, 100 U ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin and 5×10^{-5} M 2-mercaptoethanol. A control for serological specificity (not shown) was substitution of B6/Ly-1.1 (congenic) spleen cells for B6 (Ly-1.2) spleen cells; in this case pretreatment with anti-*Ly-1.2* serum and C gave no suppression of helper cell function. ○, Anti-*Ly-2.2*; ●, diluent only; ▲, anti-*Ly-1.2*.

Table 1 Cytotoxicity assays of Thy-1 and Ly antisera on various cell populations of B6 and B6/Ly-congenic mice

Serum anti-	Cells from	Titre on thymocytes (reciprocal)	Thymocytes	CRT		% Cells lysed* Lymph node cells		Spleen cells		NAPC	
				abs.	% T	abs.	% T	abs.	% T	abs.	% T
Thy-1.2	B6	10,240	>90	>90	100	66	100	32	100	39	100
Ly-1.2	B6	80	>90	75	83	57	86	27	84	29	74
Ly-2.2	B6	640	>90	55	61	43	60	21	65	22	56
Ly-1.1	B6/Ly-1.1	2,560	>90					26	81	30	77
Ly-2.1	B6/Ly-2.1	1,600	>90					10	31	8	21

CRT, cortisone-resistant thymocytes from mice given 1.5 mg of cortisone acetate intraperitoneally 24 h previously; abs, percentage of total population (absolute); % T, percentage of Thy-1⁺ cells present.

* Figures corrected for background of dead cells by the formula $[(a-b)/b] \times 100$ where a is percentage cells viable in control (reciprocal) antiserum with C, or normal mouse serum (NMS) with C (always >90%), and b is percentage cells viable in cytotoxicity assay with antiserum and C.

toneal cells (NAPC) from H-2-primed mice, and (c) graft-versus-host reactivity (GvH) of unprimed spleen cells.

(a) The results of a typical assay of helper cell activity are shown in Fig. 1. Helper function was virtually abolished by cytolysis of the SRBC-primed spleen cell population with anti-Ly-1 serum (specificity anti-Ly-1.2), whereas cytolysis with anti-Ly-2 serum (specificity anti-Ly-2.2) had no effect on helper function.

(b) In the CMC assay with peritoneal cells (NAPC) from allo-immunised (H-2-primed) mice, the effect of the two Ly antisera was reversed: There was no suppression or only minimal suppression of CMC by pretreatment with anti-Ly-1.2 and C, whereas pretreatment with anti-Ly-2.2 and C strongly suppressed this ('killer cell') function¹⁰.

(c) The GvH (Simonsen) assay differs from the two preceding functional tests in that it involves initiation as well as implementation of the immune response. Both Ly-1.2 and Ly-2.2 antisera suppressed the GvH response of adult unprimed parental spleen cells inoculated into newborn F₁ hybrid recipients, as measured by Simonsen's spleen weight index.

Thus elimination of a proportion of T cells lysed by a particular Ly antiserum does not produce equivalent losses of different T-cell functions, and different Ly antisera eliminate T cells with different functions.

In these experiments the helper population was rich in Ly-1 and poor in Ly-2, and the reverse was the case with T cells responsible for CMC. The population involved in initiation and/or implementation of GvH evidently involves T cells expressing both Ly types, or more probably T cells of two Ly categories.

Table 2 Effect of pretreatment with anti-Ly sera and C on the cell-mediated cytotoxicity (CMC) function of non-adherent peritoneal cells (NAPC) from H-2-primed donors

NAPC* donors	Pretreatment†	CMC %‡	
		Experiment 1	Experiment 2
Non-immune	Diluent only	0	0
Immune	Diluent only	50	76
Immune	NMS + C	64 (Standard)	78
Immune	Anti Ly-1.2 + C	65	67
Immune	Anti Ly-2.2 + C	28	30

* B6 donors received a single intraperitoneal injection of 10⁸ BALB/c (immune) or B6 syngeneic (non-immune control) spleen cells in Experiment 1 and multiple intraperitoneal injections of 5 × 10⁶ BALB/c (immune) or B6 syngeneic (non-immune) spleen cells in Experiment 2. NAPC were collected 5 d after an injection in Experiment 1 and 3 d after the last injection of four consecutive weekly injections in Experiment 2.

† See footnote Fig. 1.

‡ ³H-proline CMC micro-assay^{13,14} in which the radioactivity of surviving attached monolayer target cells is measured. Washed NAPC (pretreated as in Fig. 1; 1 × 10⁵ per well) were applied to a ³H-proline-labelled monolayer of BALB/c Meth A sarcoma cells (1 × 10⁵ per well) for 14 h. CMC % was calculated from the c.p.m. of the washed surviving monolayers after exposure to (a) Immune NAPC or (b) non-immune (control) NAPC; according to the formula $[1-(a/b)] \times 100\%$.

Table 3 Effect of pretreatment with different Ly antisera and C on the GvH capacity of spleen cells in the Simonsen assay¹⁵

Pretreatment of spleen cells*	Spleen indices of individual recipients†	Mean
None	1.98, 1.78, 2.03, 2.18 2.82, 2.21, 2.91, 3.28 1.33, 1.67, 1.80, 1.08	2.44
Anti-Ly-1.2 + C	0.95, 1.09, 1.14, 1.13 1.50, 1.21, 1.46, 1.75	1.27
Anti-Ly-2.2 + C	1.42, 1.42, 2.67, 1.57 1.81	1.64

* See legend to Fig. 2.

† 1 × 10⁶ normal B6 spleen cells (pretreated as in Fig. 2) were injected intraperitoneally into 1-5-d-old (B6 × BALB/c) F₁ hybrids. Spleen weight indices were calculated 9 d later according to Simonsen.

In addition to their implication with regard to the diversity of T cells, our findings suggest a means of isolating T-cell subsets with particular functions according to their Ly profiles. At the moment this can be said safely only of the B6 mouse with which these experiments were conducted; it remains to be seen whether the Ly profiles established for T-cell functions of this strain will prove to be representative of all mice.

This work was supported by a WHO fellowship and the Garrett Fund, Memorial Hospital (P.K.), and by grants from the Cancer Research Institute, Inc. (H.S.) and the National Cancer Institute.

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Received September 24; revised November 29, 1974.

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